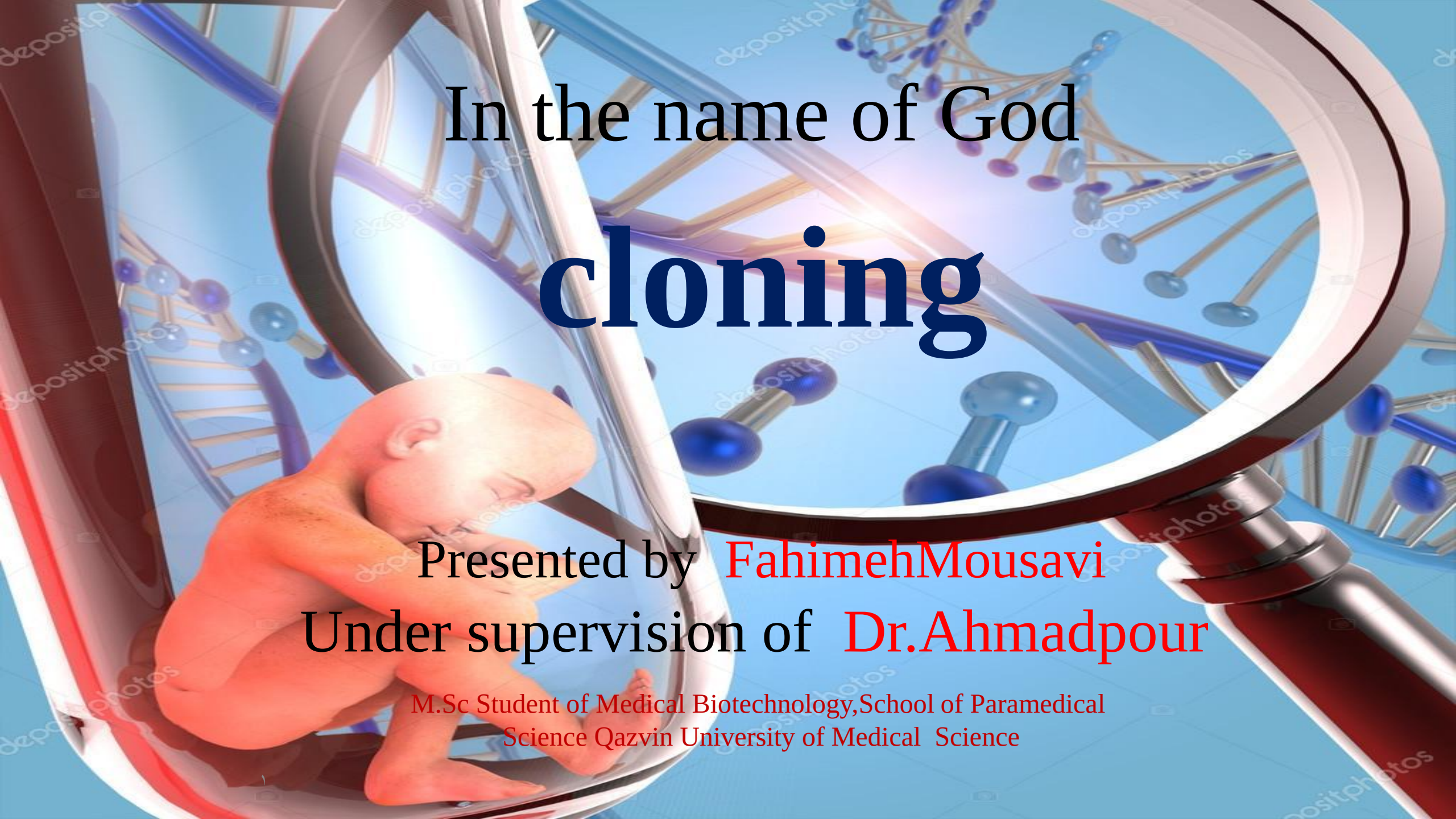


In the name of God

cloning

Presented by FahimehMousavi

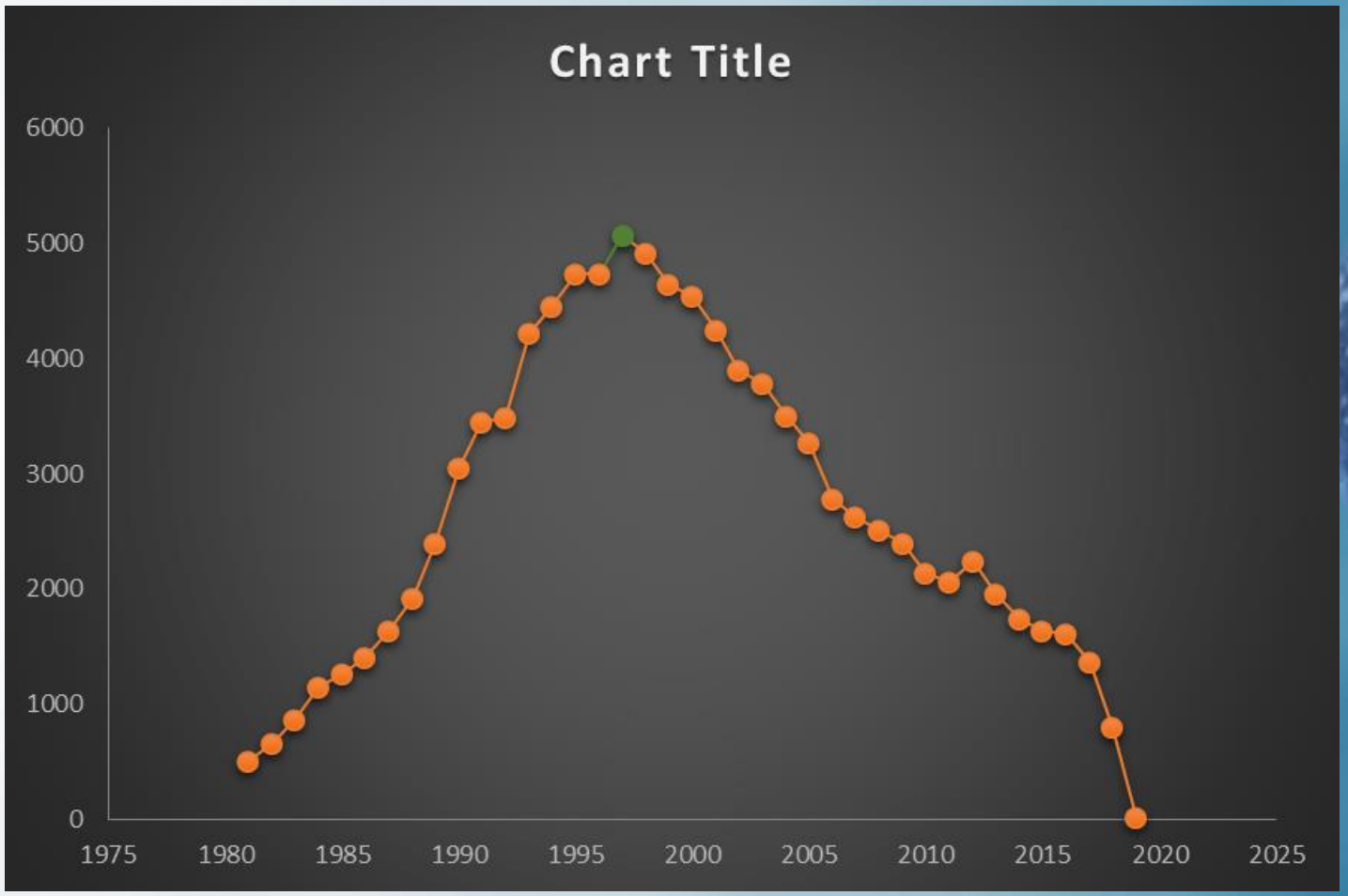
Under supervision of Dr.Ahmadpour

M.Sc Student of Medical Biotechnology,School of Paramedical Science Qazvin University of Medical Science


All About
Cloning

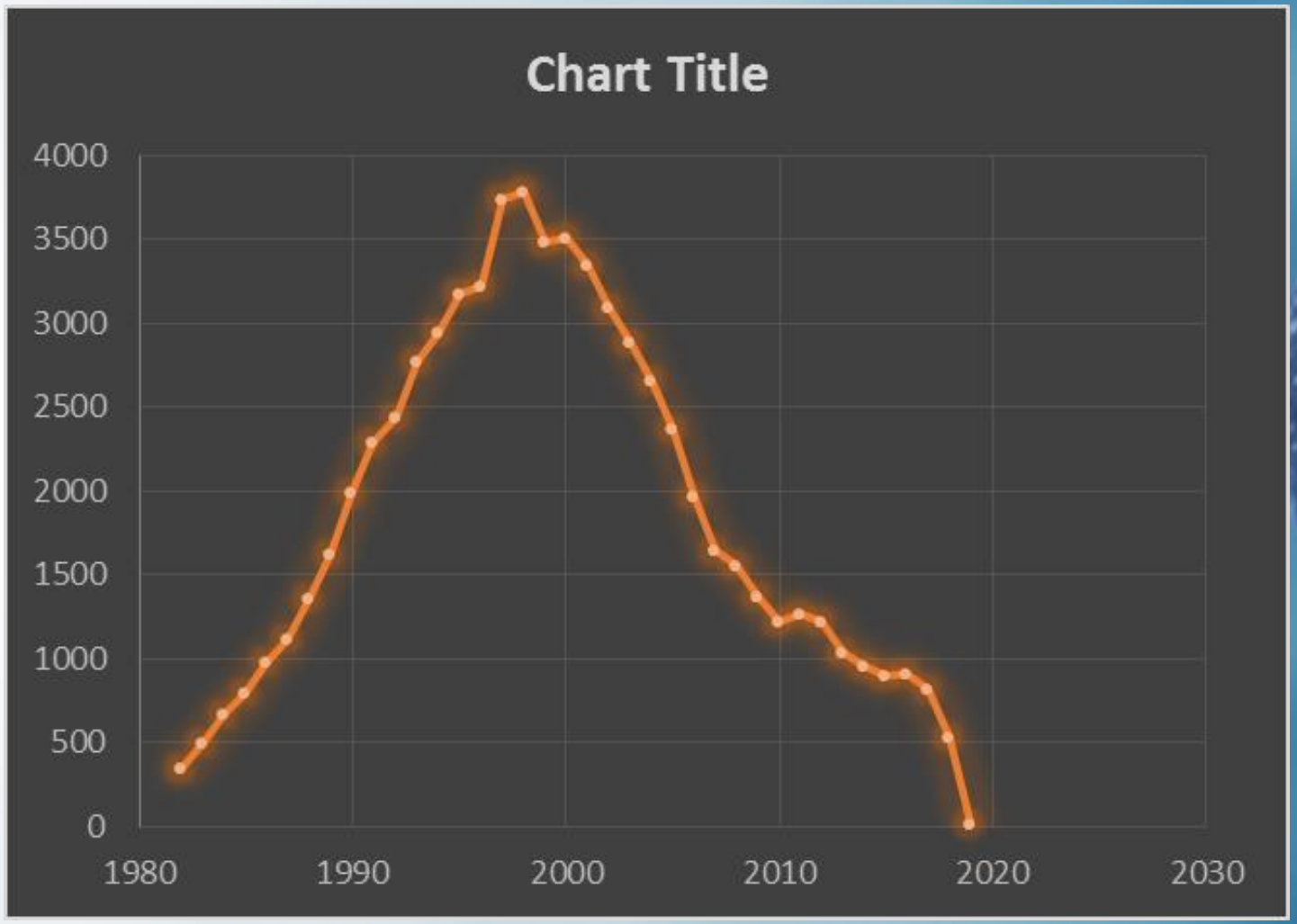
Animal cloning1980-2019

Pubmed-1January 20019



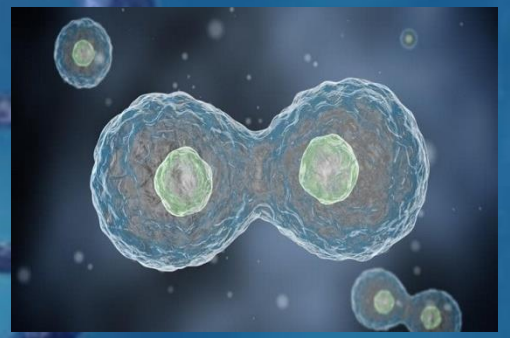
Human cloning1980-2019

Pubmed-1January 20019



What is cloning ?

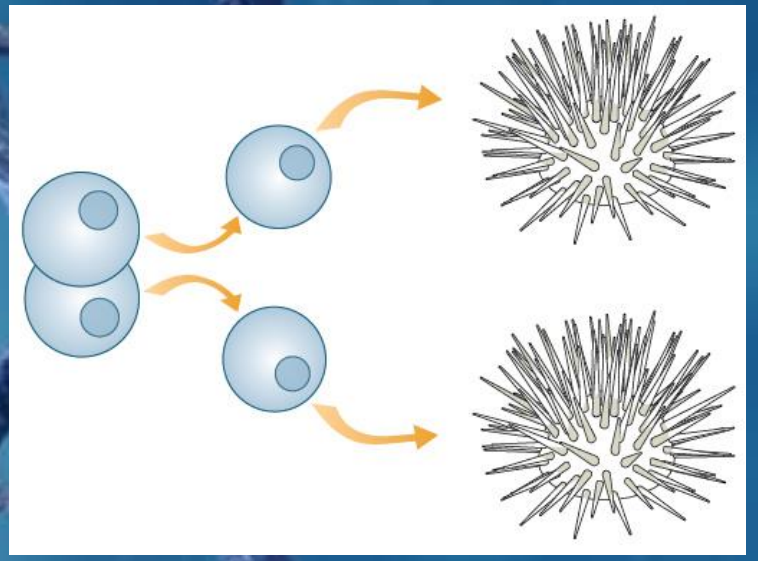
- Cloning means making an organism identical to another
- In nature; Identical twins
- Occurs naturally in single celled organisms
- Cutting plant



The history of Cloning

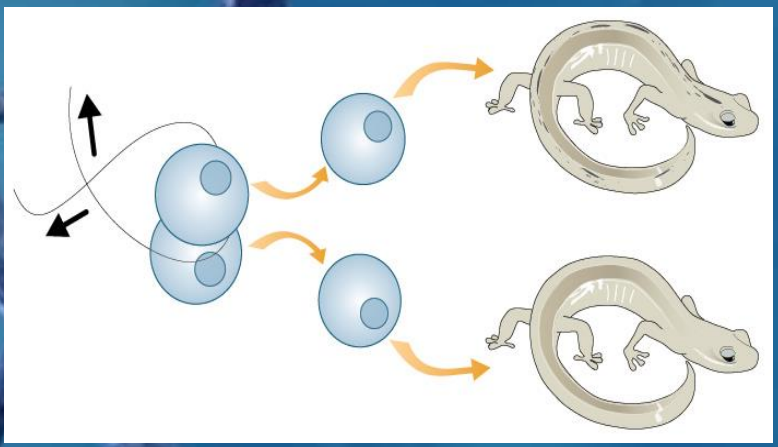
1885- first- ever demonstration of artificial embryo twinning.(sea urchin)

Hans Adolf Eduard Driesch



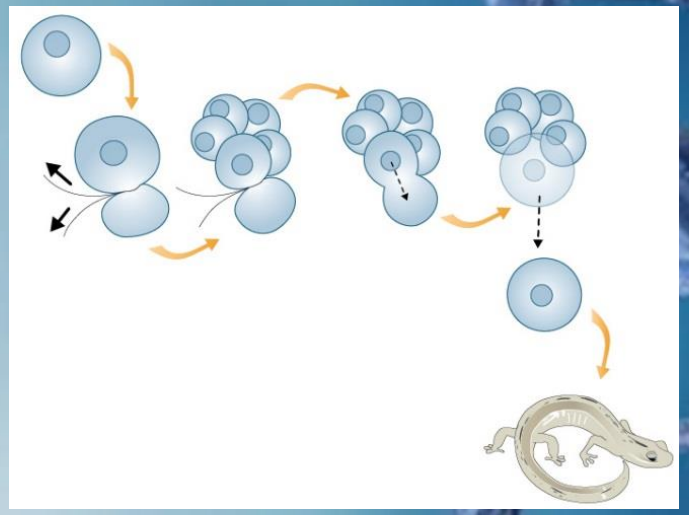
1902 – artificial embryo twinning in a vertebrate. (Salamander)

Hans Spemann



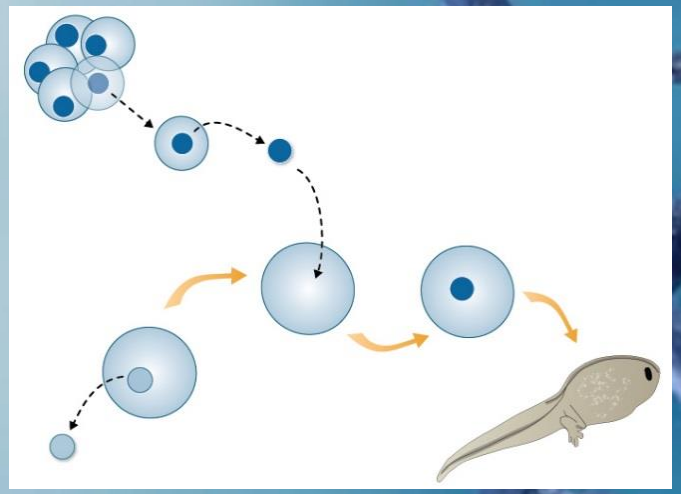
1928-The cell nucleus controls embryonic development. (Salamander)

Hans Spemann



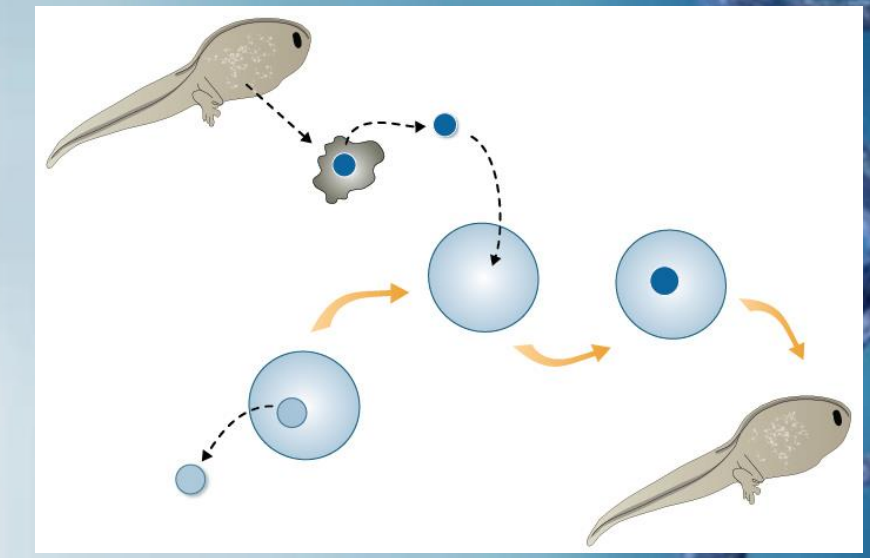
1952- First successful Nuclear transfer. (Frog)

Robert Briggs and Thomas king



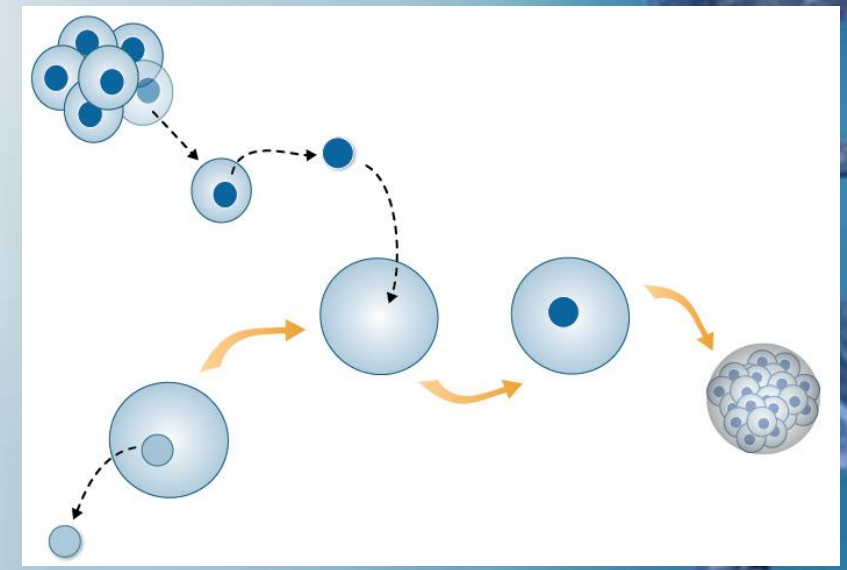
1958-nuclear transfer from a differentiated cell.(Frog)

John Gurdon



1975-First mammalian embryo created by nuclear transfer.(Rabbit)

J. Derek Bromhall



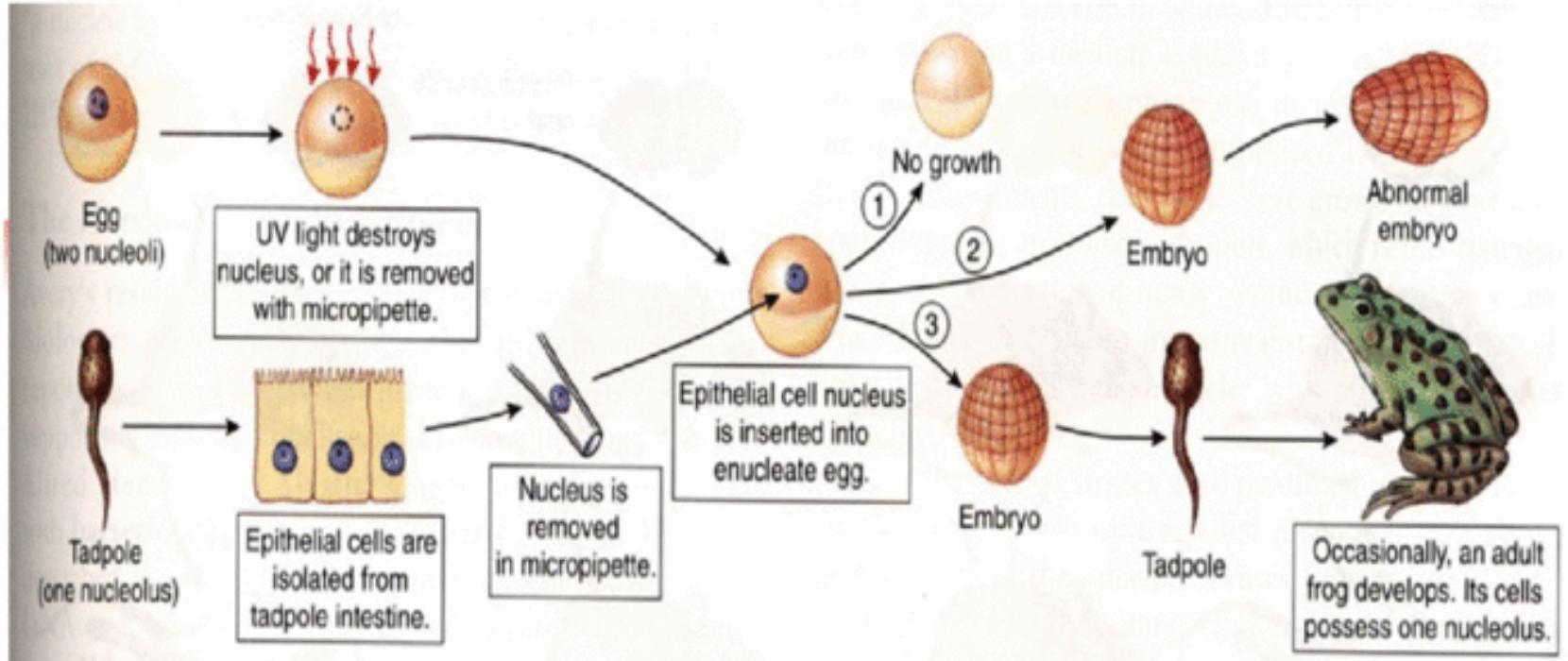
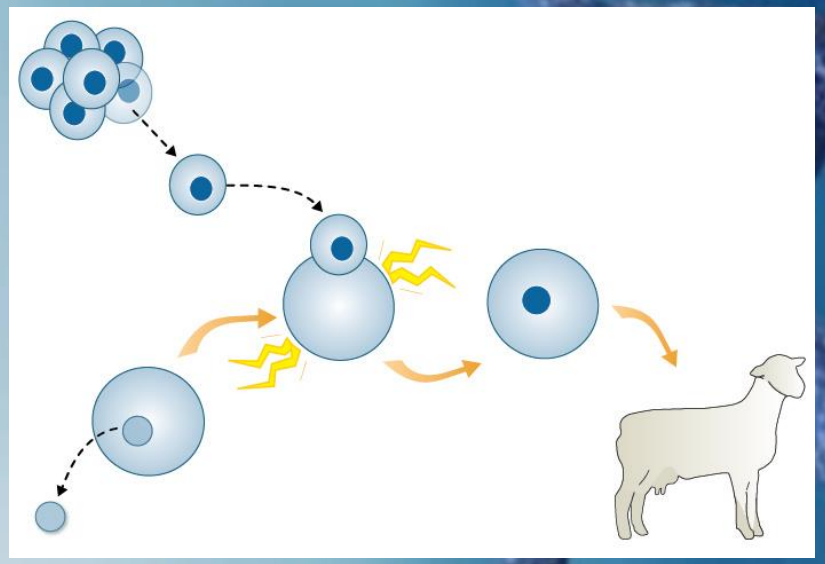


FIGURE 14.3

Briggs and King's nuclear transplant experiment. Two strains of frogs were used that differed from each other in the number of nucleoli their cells possessed. The nucleus was removed from an egg of one strain, either by sucking the egg nucleus into a micropipette or, more simply, by destroying it with ultraviolet light. A nucleus obtained from a differentiated cell of the other strain was then injected into this enucleate egg. The hybrid egg was allowed to develop. One of three results was obtained in individual experiments: (*1*) no growth occurred, perhaps reflecting damage to the egg cell during the nuclear transplant operation; (*2*) normal growth and development occurred up to an early embryo stage, but subsequent development was not normal and the embryo did not survive; and (*3*) normal growth and development occurred, eventually leading to the development of an adult frog. That frog was of the strain that contributed the nucleus and not of the strain that contributed the egg. Only a few experiments gave this third result, but they serve to clearly establish that the nucleus directs frog development.

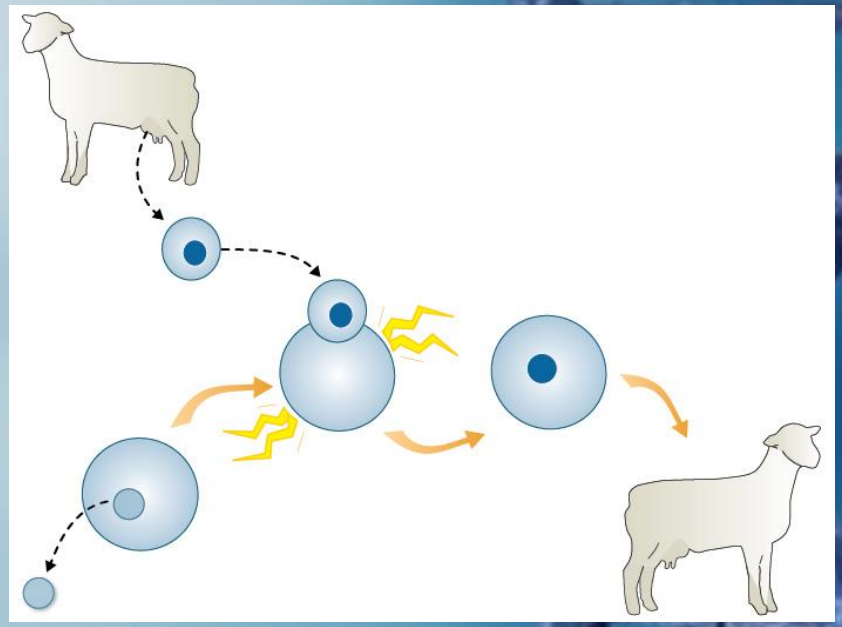
1984-First mammal Created by nuclear transfer.(Sheep)

Steen Willadsen



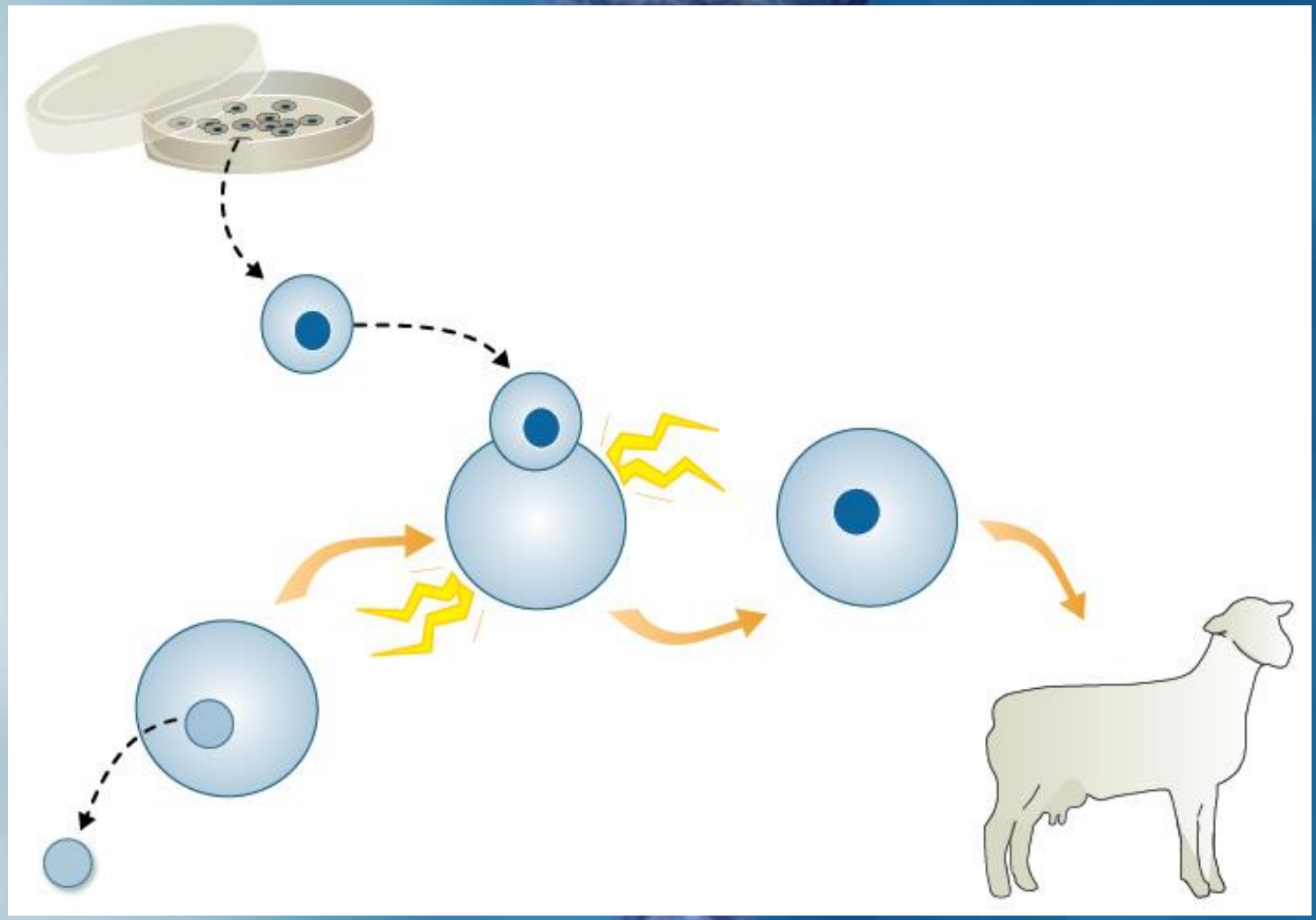
1996-Dolly First mammal created by somatic cell nuclear transfer.(sheep)

Ian Wilmut and Keith Campbell



1996-nuclear transfer from laboratory cells(sheep)

Lan Wilmut and Keith Campbell



Cloning for medicine

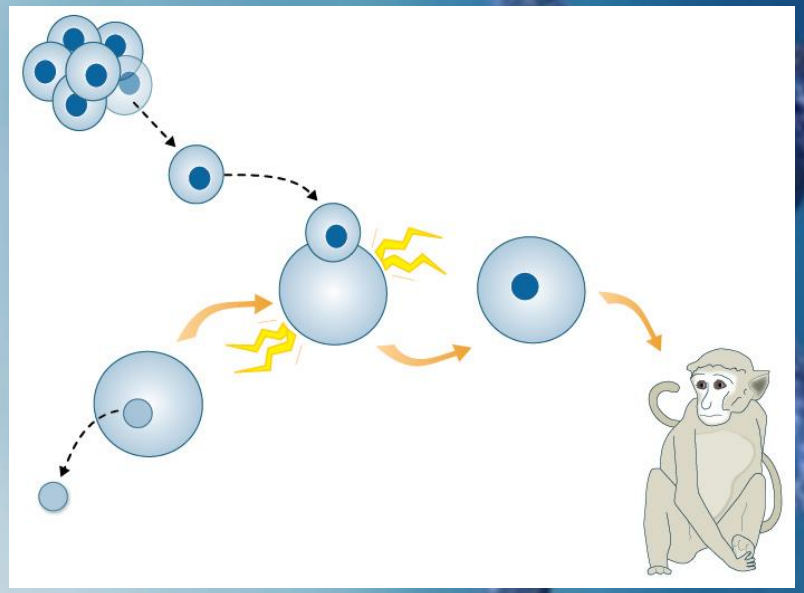
In 1996 ;scientists at Roslin institute near Edinburgh ;Scotland ; managed to produce Megan and Morage from cultured cells derived from a nine-day-old embryo.

This made Megan and Morage clones of the embryo.

MEGAN AND MORAG (*above*) were the first mammals cloned from cultured cells. That basic technique has allowed the creation of cloned sheep carrying human genes. Such animals produce milk that can be collected and processed (*left*) to yield therapeutic human proteins.

1997-First primate created by embryonic cell nuclear transfer.(Rhesus monkey)

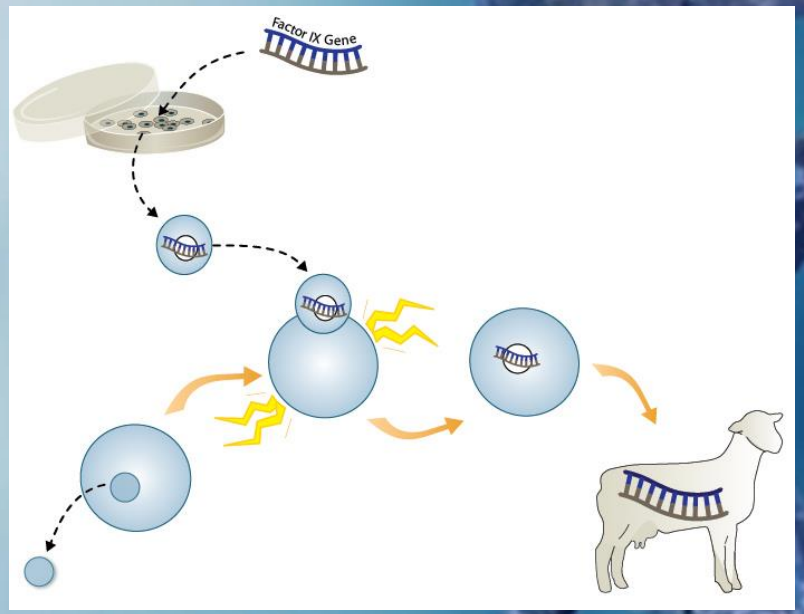
Li Meng , John Ely, Richard Stouffer, and Don Wolf



Neti and Ditto

1997- Nuclear transfer from genetically engineered laboratory cells.(sheep)

Angelika Schnieke , keith Campbell , lan Wilmut

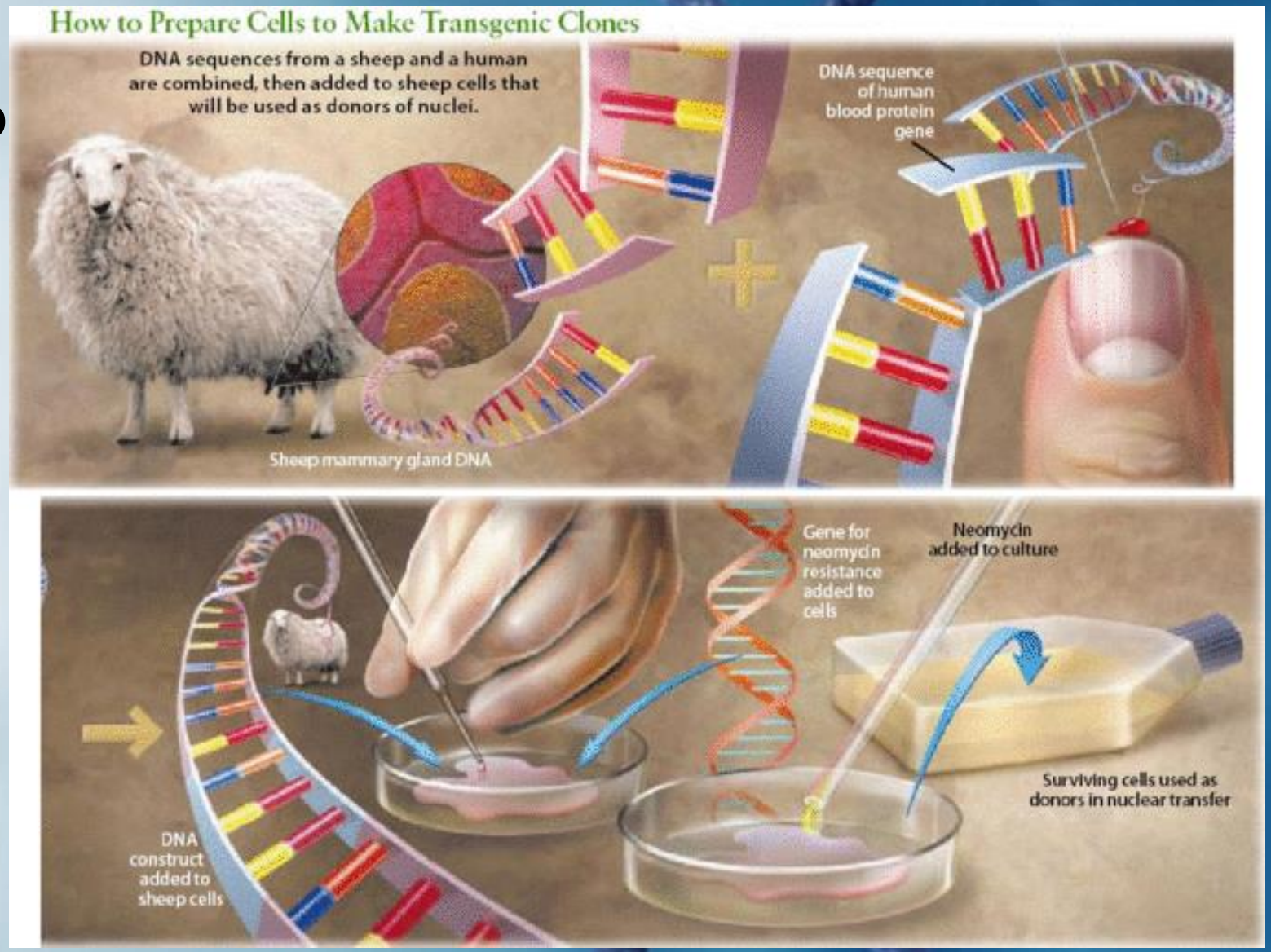


Polly

(left)is a transgenic clone of a poll Dorset sheep .a gene for a human protein factor ; lX; was added to the cell that provided the lamb’s genetic heritage ;so Polly has the human gene .the ewe that carried Polly (right)is a Scottish blackface

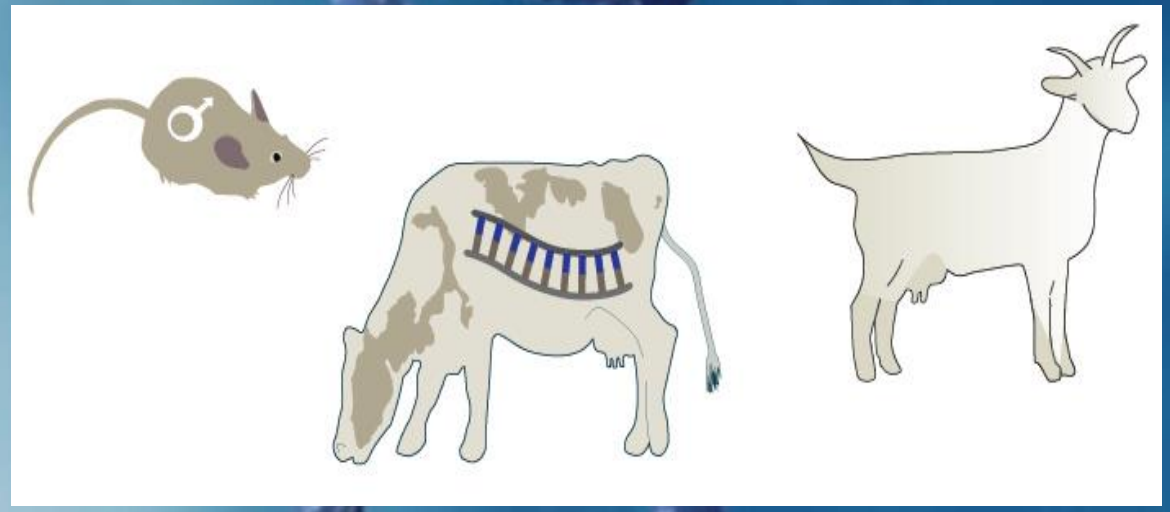
Cloned with a genetically modified cell

The first transgenic sheep was born in1997;polly and others secrete the human protein in their milk



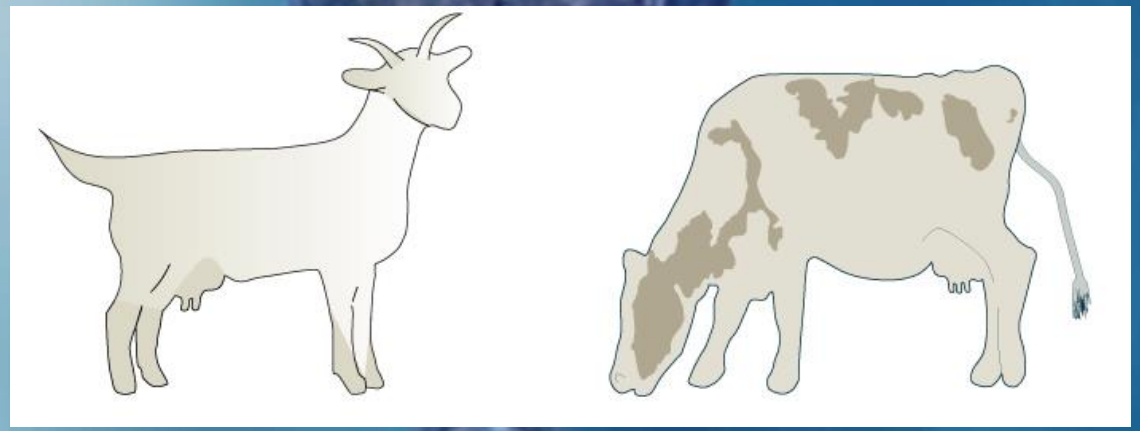
1998-1999- More mammals cloned by somatic cell nuclear transfer.(Mice , cows , and goats)

Multiple groups

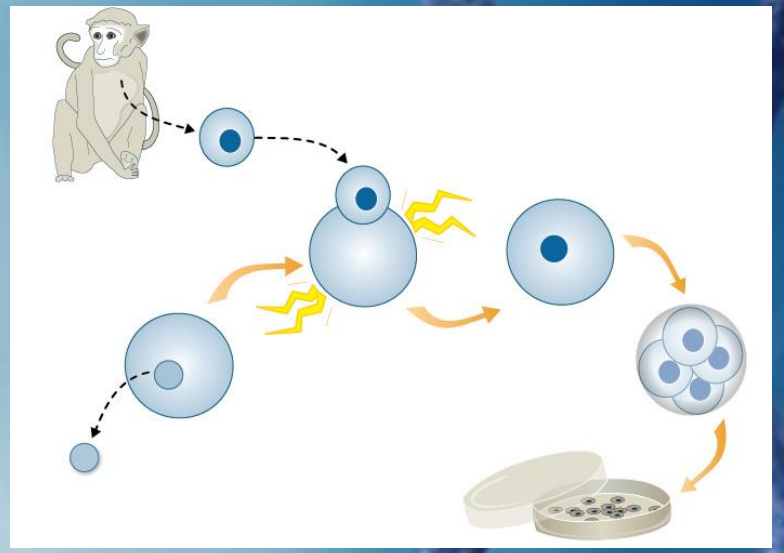


2001-Endangered animals cloned by somatic cell nuclear transfer.(Gaur and Mouflon)

Multiple groups

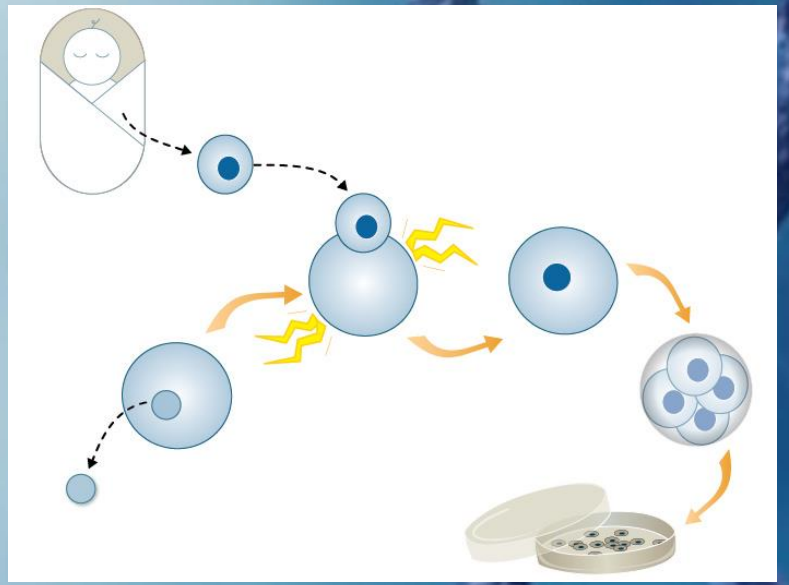


2007- primate embryonic stem cells created by somatic cell nuclear transfer.(Rhesus monkey)



Shoukhrat Mitalipov and colleagues

2013-Human embryonic stem cells created by somatic cell nuclear transfer.(Human)



Shoukhrat Mitalipov and colleagues

Types of Cloning

1.Embryo cloning

2.Reproductive cloning

3.Therapeutic Cloning

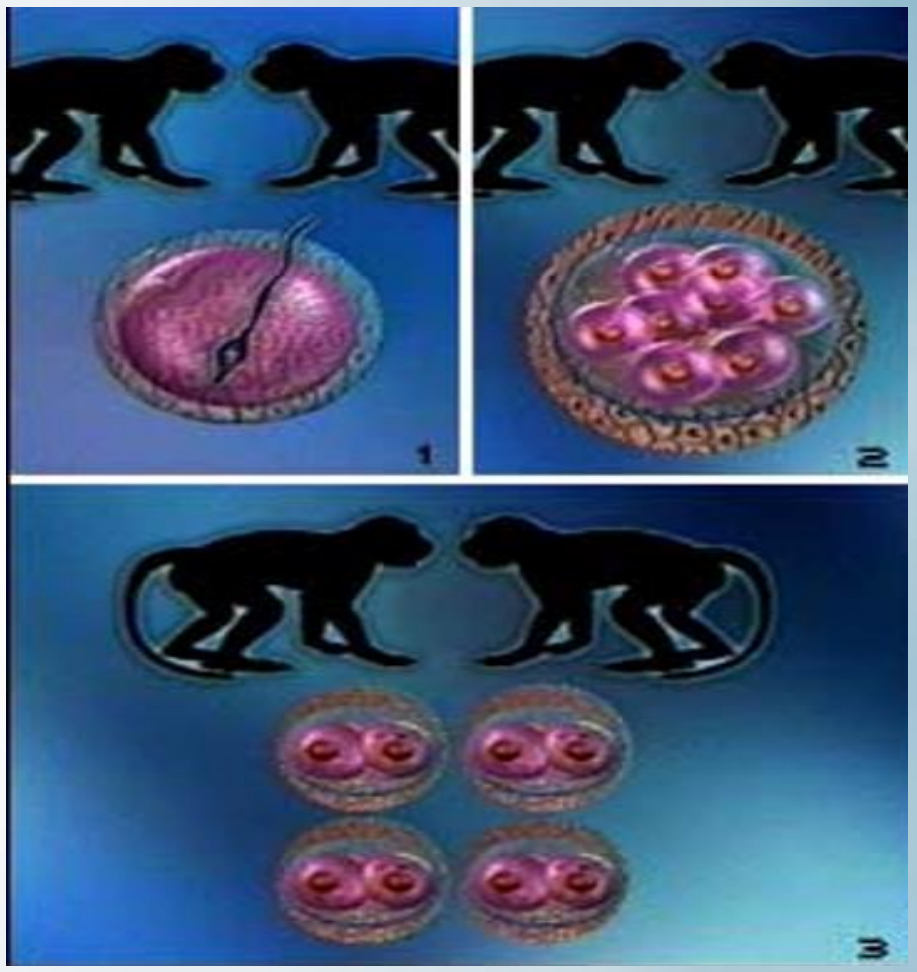
Embryo cloning

An egg from a mother and sperm from a father are used to create a fertilized egg.

After the embryo grow into eight cells , researcher split it into four identical embryos ,each consisting of just two cells.

The four embryos are then implanted into surrogate mother.

Embryo cloning



Reproductive cloning

- Produce a duplicate of existing animal
- used to clone sheep & other mammals
- Produce several genetic defects
- Medical ethicists-immoral procedure to be done on humans as it is unsafe & unethical

Therapeutic cloning

DNA is extracted from a human's cell

↓

Inserted into a woman's ovum

↓

Develop and produce stem cell

↓

Stem cells are removed from the pre-embryo

↓

Grown into specific organ

↓

Transplanted into the patient

Direct advantages of cloning

- Cloning animals for disease assessment
- Cloning Stem cells for research
- Drug preparation
- Protecting endangered species

Value of therapeutic cloning for patient

Potential application in commonly occurring diseases:

- Heart diseases
- Insulin dependent diabetes
- Parkinson diseases
- Burns

In direct advantages of cloning

- First , inducing cancer cells to differentiate
- Second , aged cell nuclei can be rejuvenated.
- In aged frog red blood cell nuclei after their transfer into enucleated oocytes , frog red blood cell nuclei were rejuvenated.

Problem of cloning

1)Expensive

2)The hope of doing it is desirable

3)Abnormal growth of clones

Abnormal growth of clones

1)Abnormal enlargement of the embryo

2)Increase the length of pregnancy

3)Weakness of the baby

4)Death

5)Infectious disease

History of SCNT

- ✓ IN1894,Jacques Loeb observed formation of a large bleb in early embryos

- ✓ he found that if nucleus moved into the bleb , this part of the embryo started to develop

History of SCNT

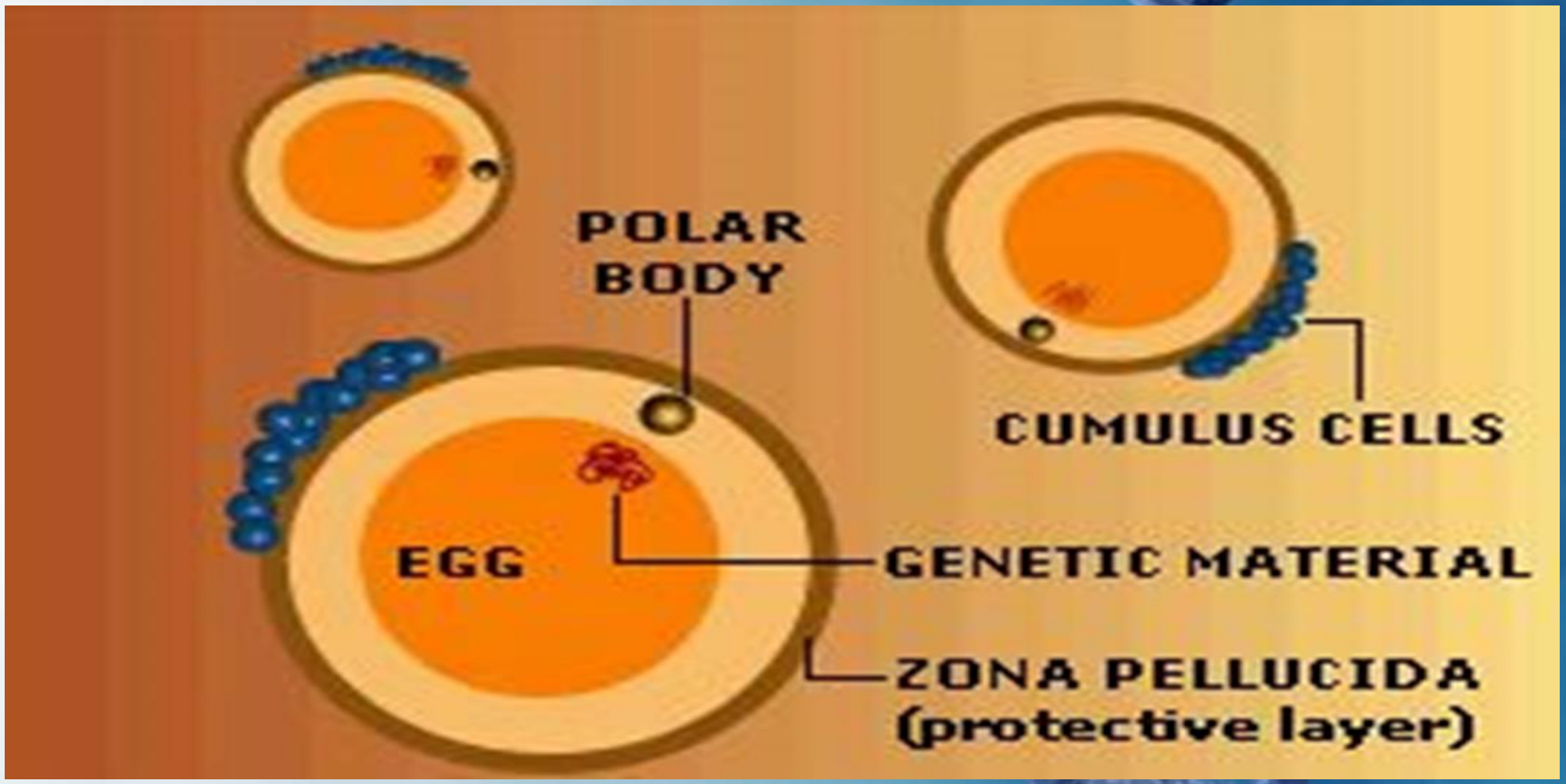
- ✓ Loeb's roads were furthered by Yves Delage (1895) & Hans Spemann (1936)

- ✓ Nuclear transfer experiments from sea urchins to tadpoles & from mice to sheep(Wilmut in). 2003) and finally to primates(Hwang woo- suk in 2005 & Mitalipov in 2013

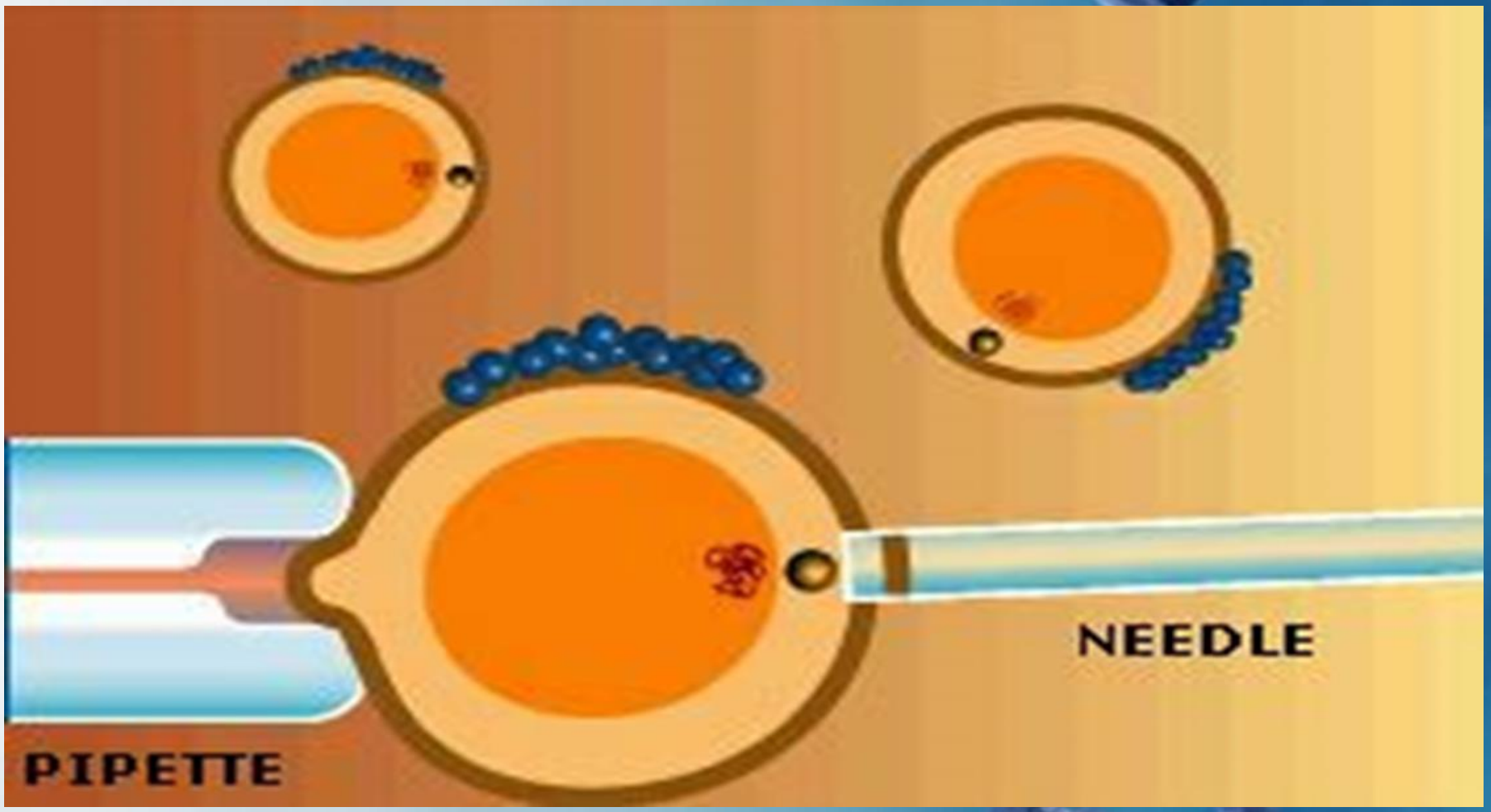
Technology behind Therapeutic cloning

- Removing nucleus of egg cell
- Replace with nucleus of a somatic cell
- Stimulate cell division

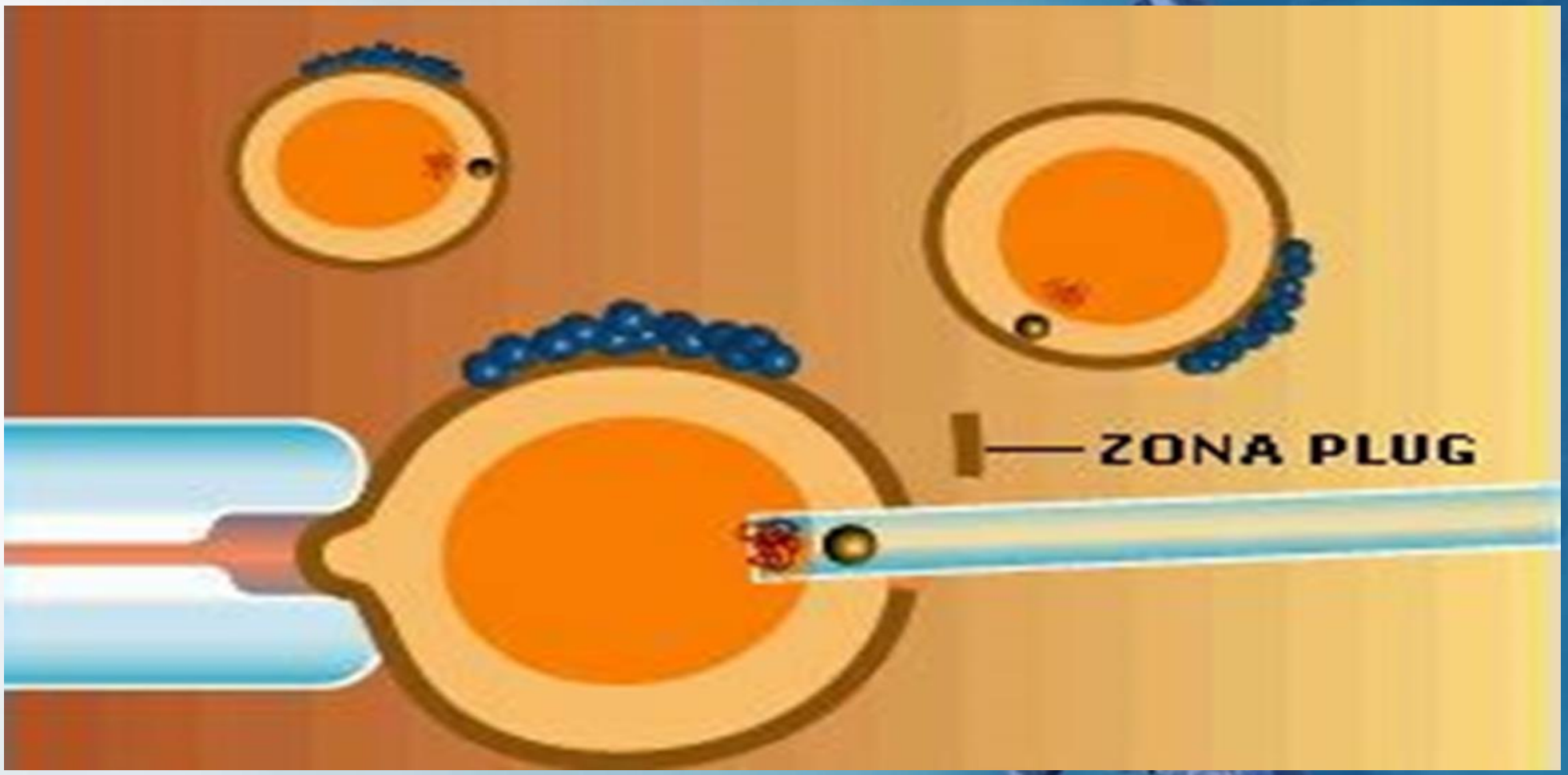
How is Therapeutic Cloning Done ?



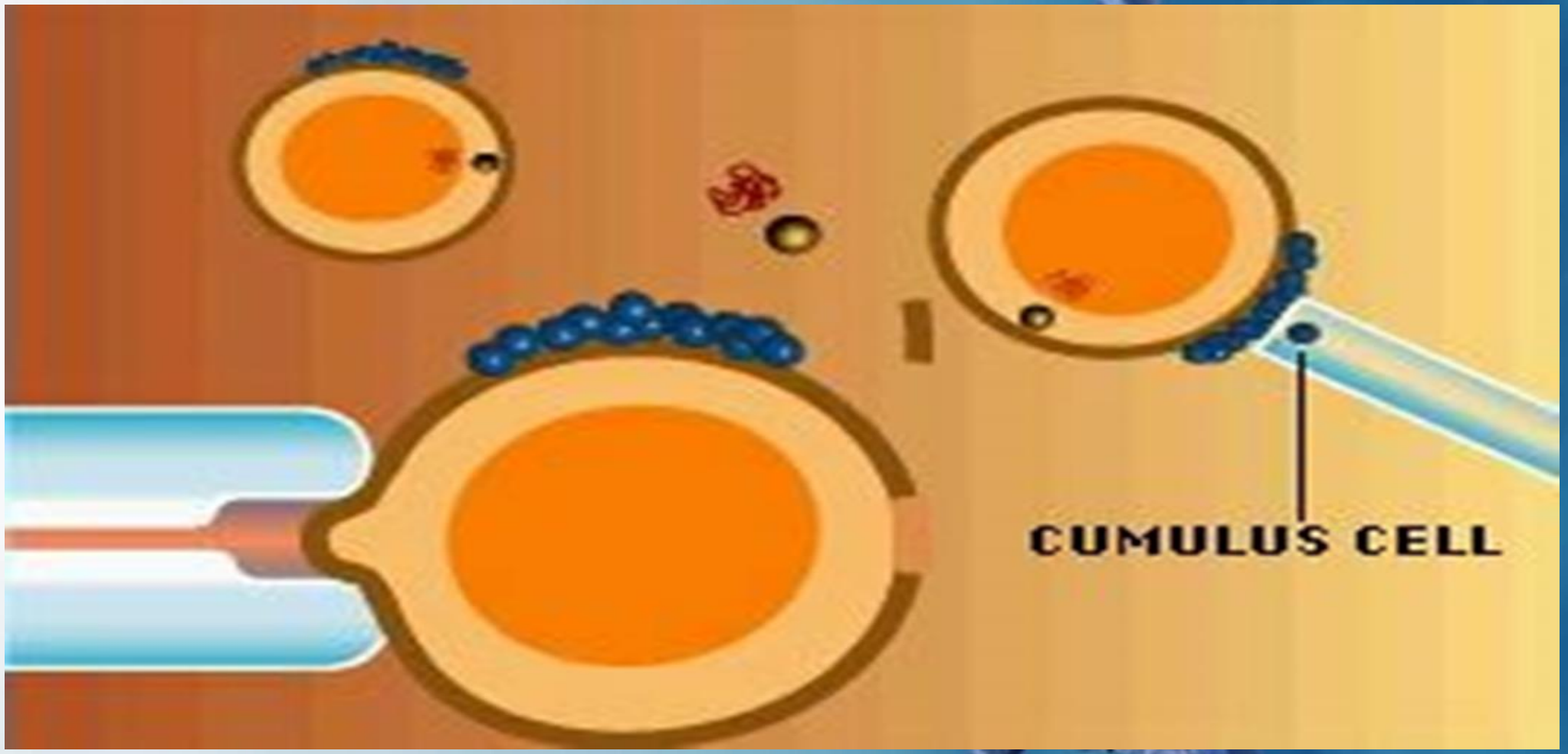
While an egg is held still with a pipette, a needle is used to drill through zona pellucida, removing a plug.



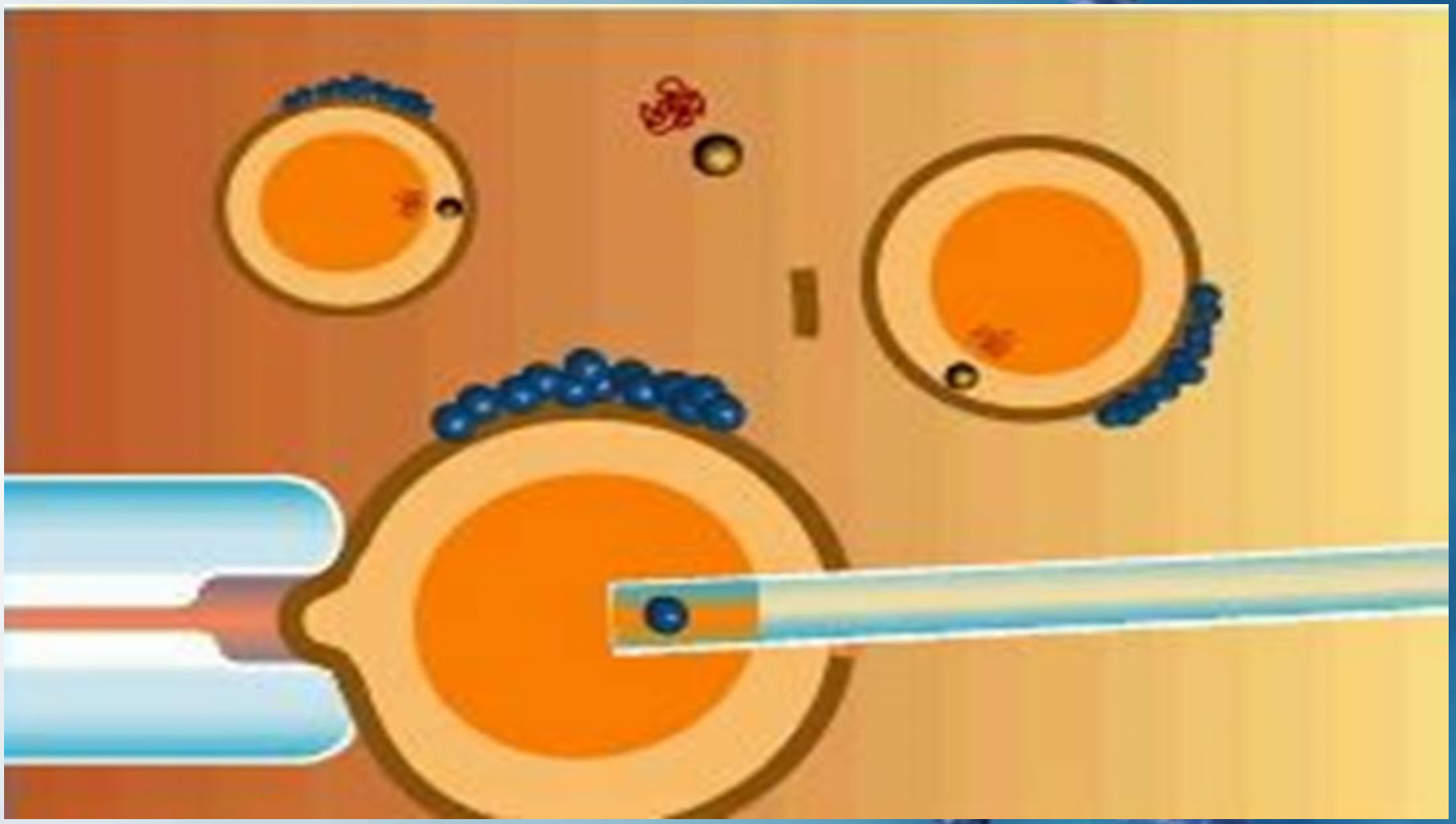
After ejecting the zona plug the needle is inserted back in the egg through the hole to withdraw and discard the polar body and the egg's genetic material



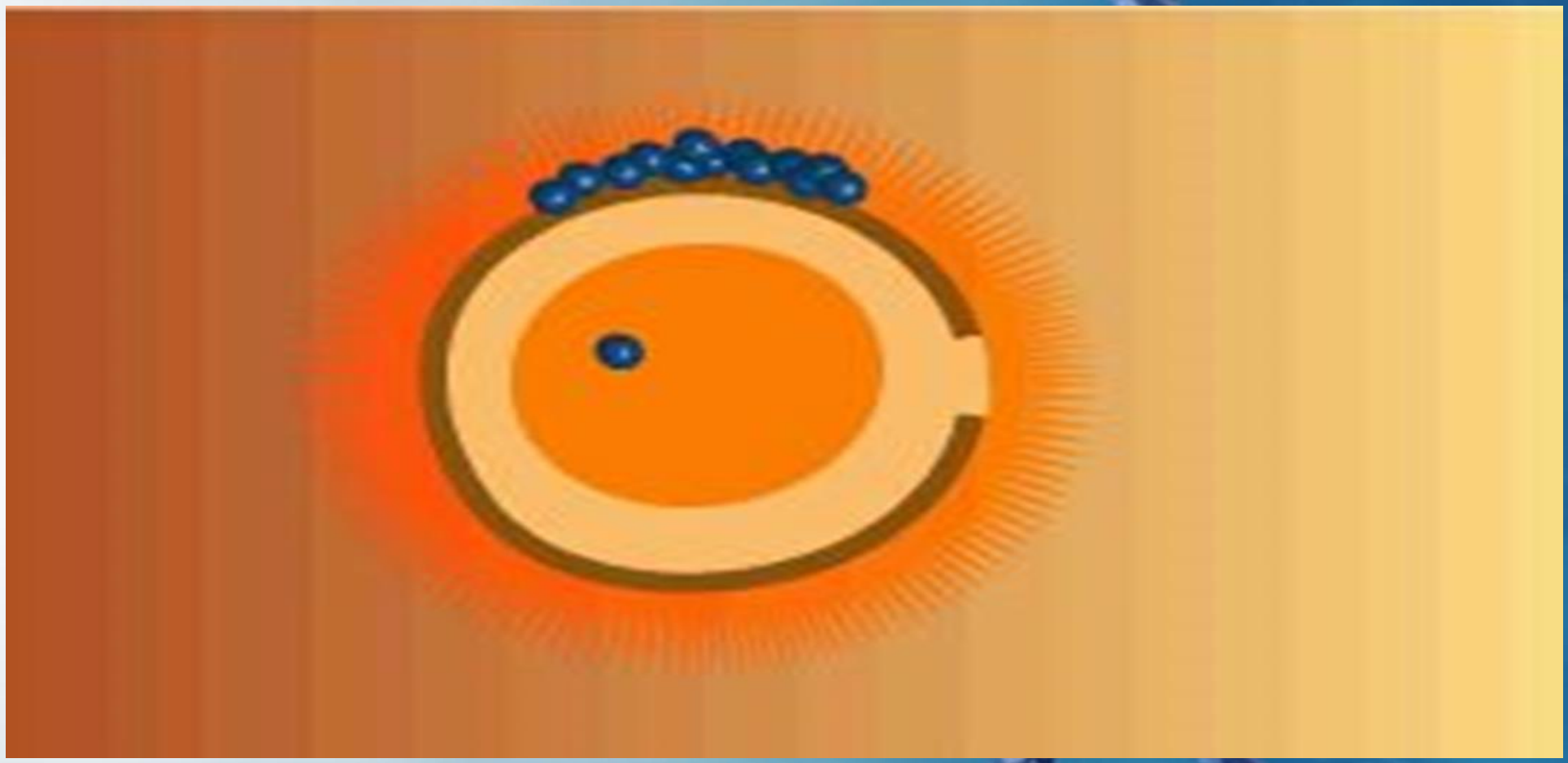
A cumulus cell from another egg is taken up into the needle. Cells called fibroblasts (or their nuclei) can also be used in this step.



The cumulus cell is injected deep into the egg that has been stripped of its genetic material.

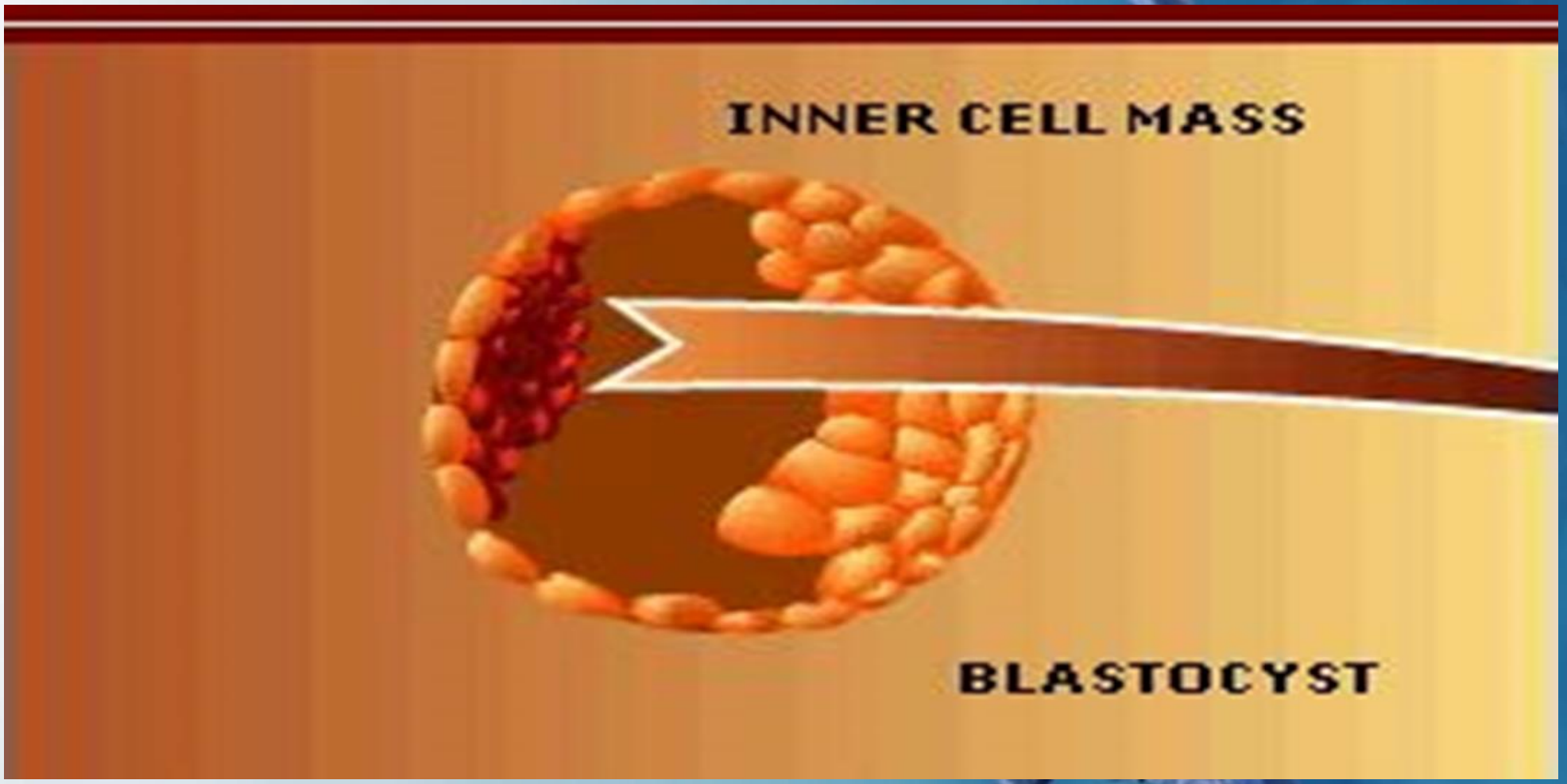


The injected egg is exposed to a mixture of chemicals and growth factors designed to activate it to divide.

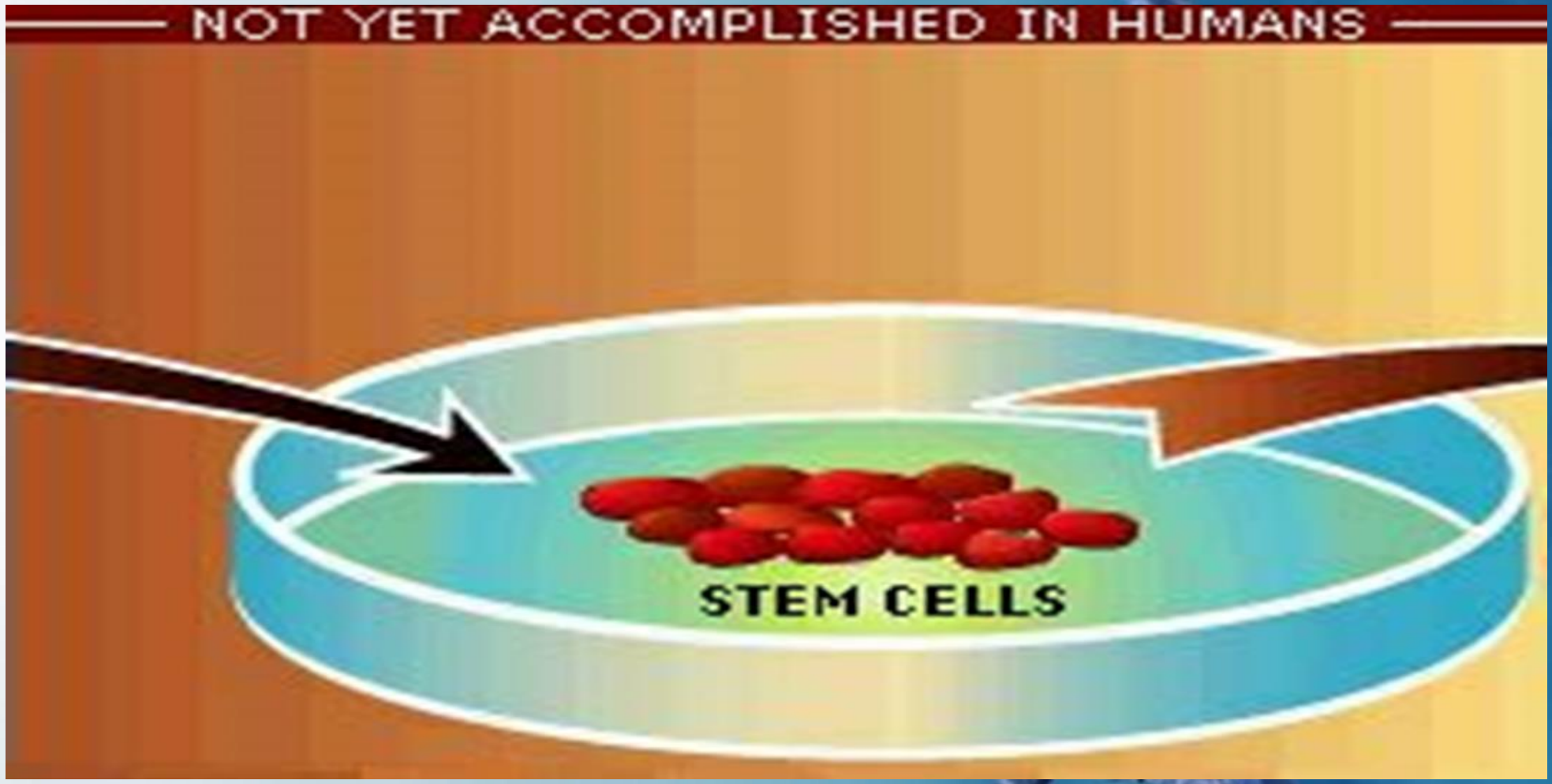


After roughly 24 hours , the activated genetic egg begins dividing. The cells contain material only from the injected cumulus cell.

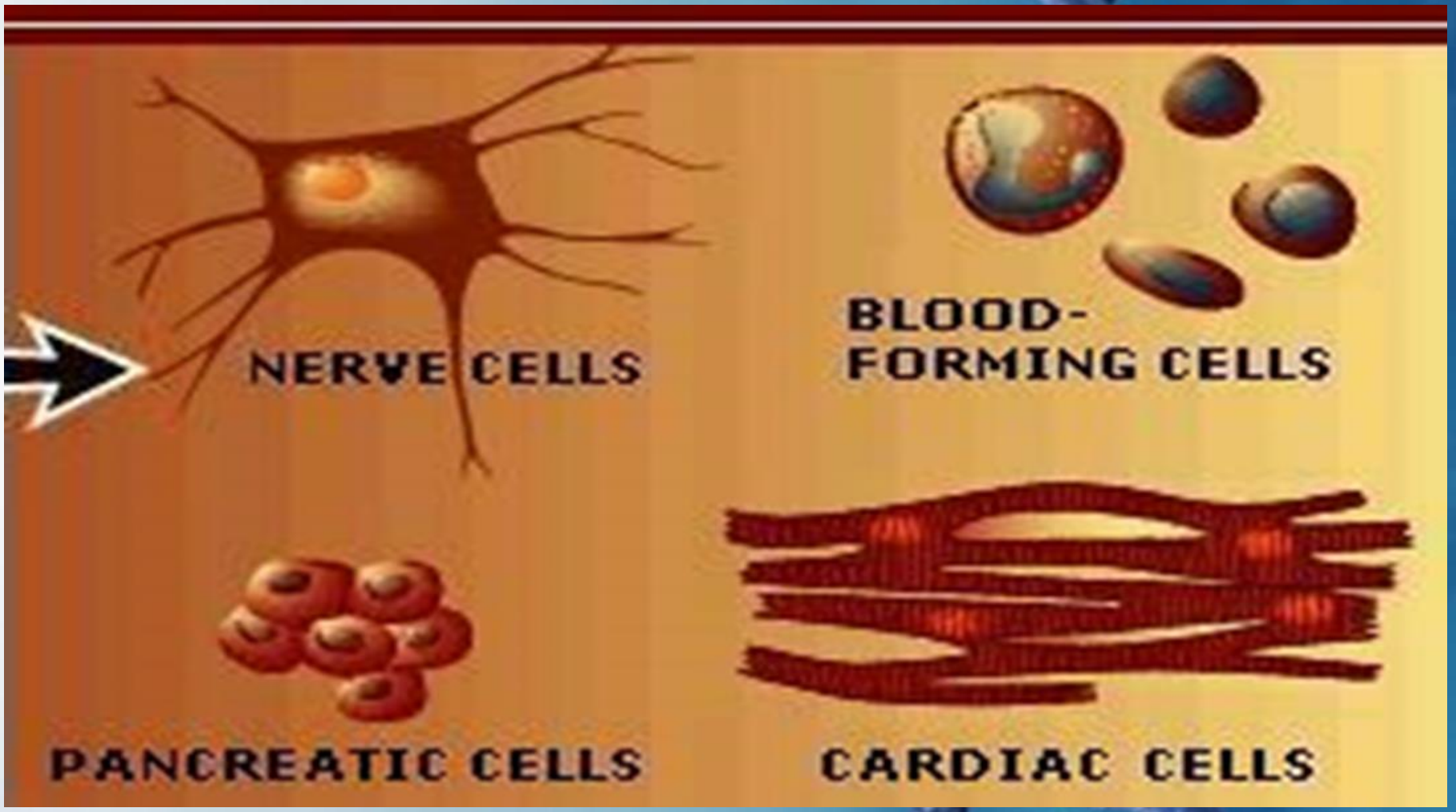
By the fourth or fifth day, a hollow ball of roughly 100 cells has formed. It holds a clump of cells called the inner cell mass that contain stem cells.



The blastocyst is broken open, and the inner cell mass is grow in a culture dish to yield stem cells.



The stem cells , in turn, can be coaxed to grow into a variety of cells that might one day be injected into patients.



Cloning Techniques

1.Roslin technique

2.Honolulu technique

Roslin technique

- ✓ Is a variation of SCNT that was developed at the Roslin institute
- ✓ Is used to create Dolly
- ✓ Somatic cells are allowed to grow and divide and are deprived nutrients to induce the cells into a suspended or dormant stage.

Roslin technique

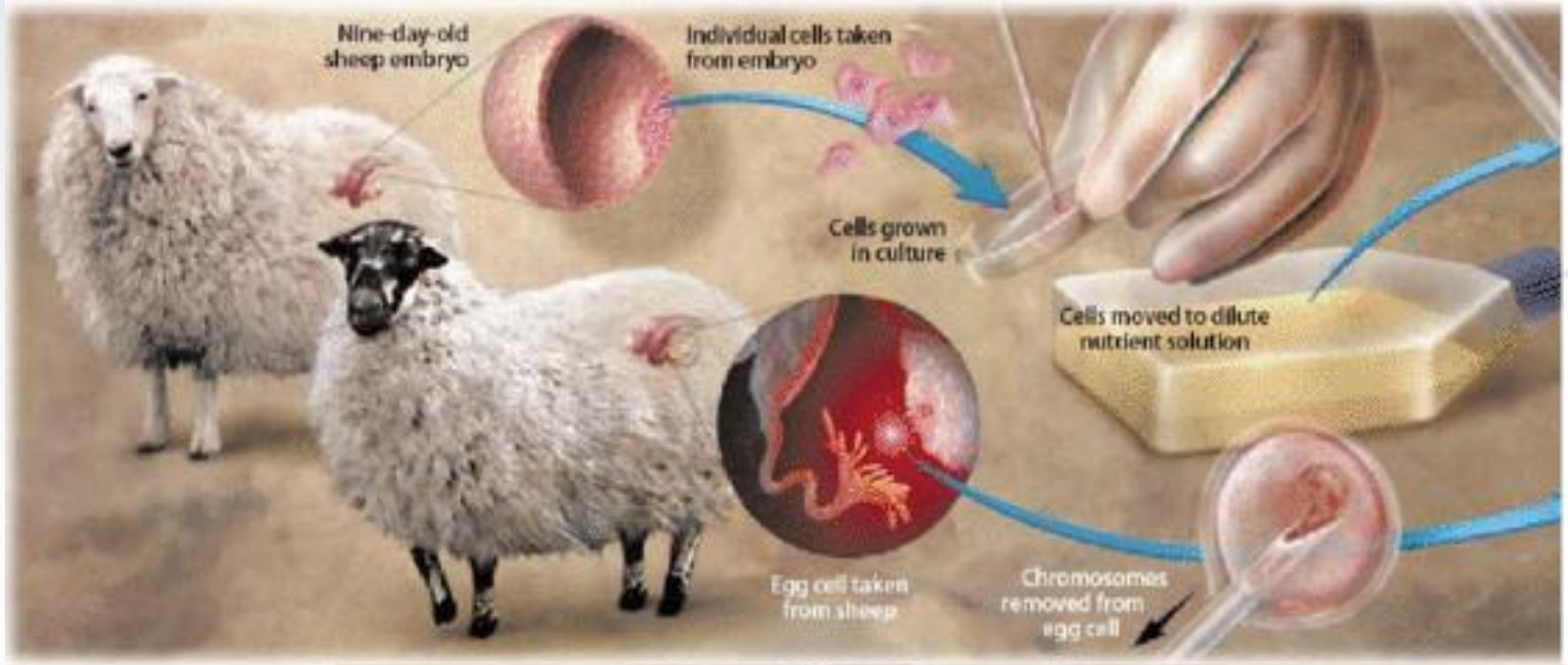
- ✓ An egg cell that has had its nucleus removed is then placed in close proximity to a somatic cell and both cells are shocked with an electrical pulse.
- ✓ The cells fused and the egg is allowed to developed into an embryo.
- ✓ The embryo is then implanted into a surrogate.

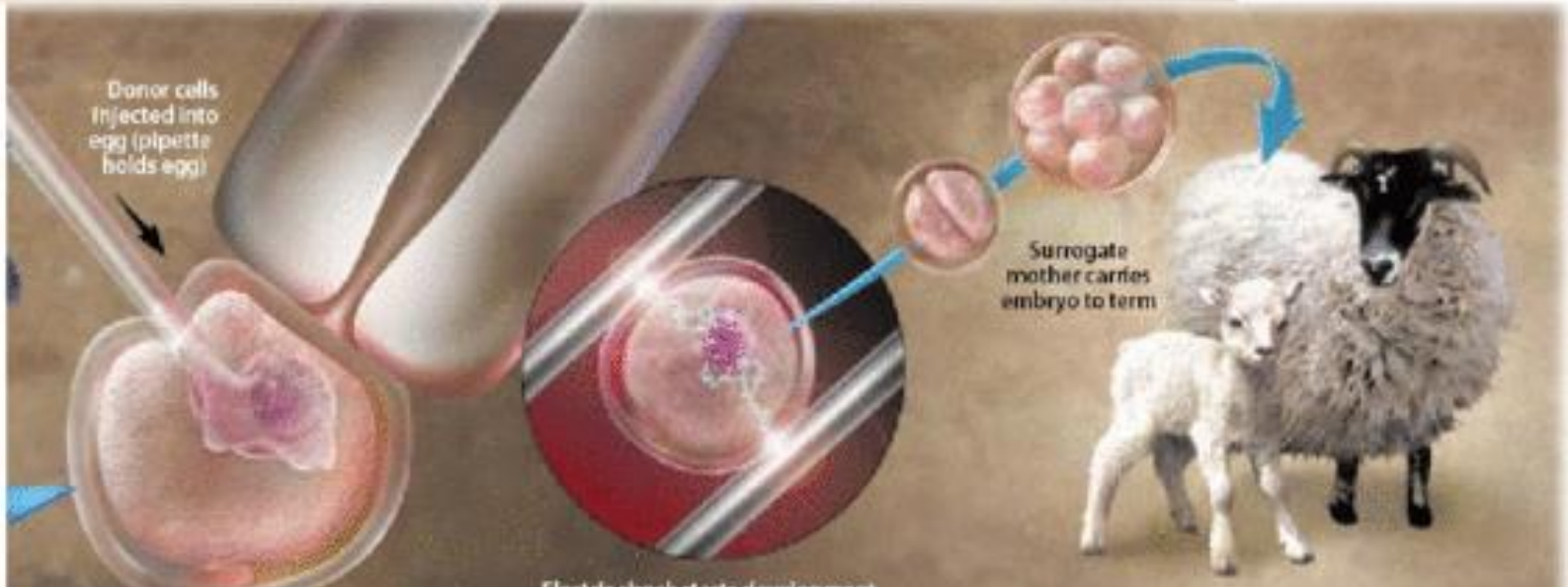
Honolulu technique

- Developed at University of Hawaii , Honolulu
- Does not electric charge for cell fusion
- Fused cell- given a chemical bath
- Three donor cells – sertoli ,neuronal &cumulus cells

How Megan and Morag Were Made

Cultured cells were combined with egg cells to yield embryos that developed into cloned offspring.





Dolly is only the first

Gender: female

Breed : Mammal

Birthday : 1996 /7/ 5

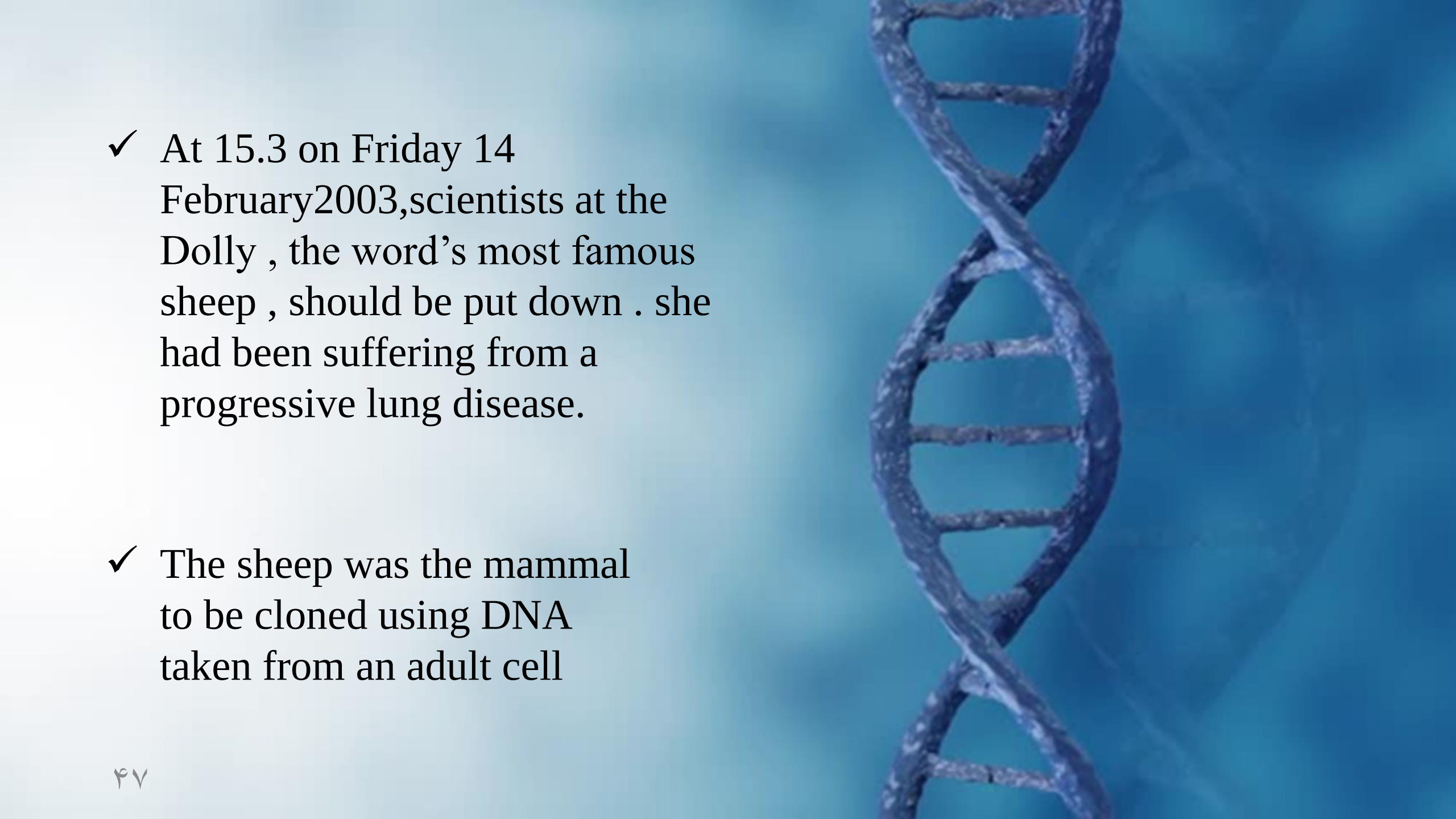
Home place: Scotland

Father: no father

Mother: Three mother

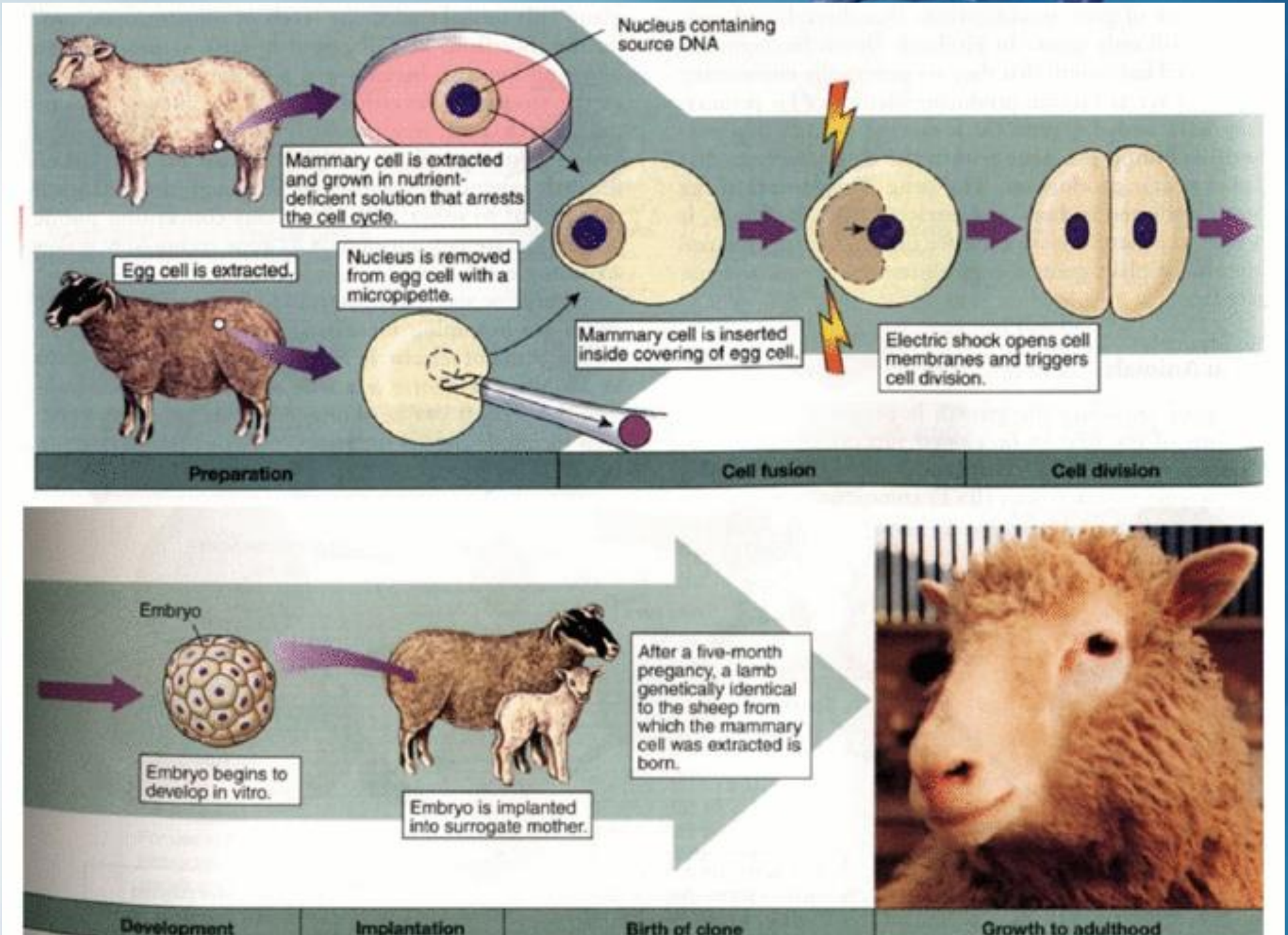
Family: 5kid

Death: 2003/2/14



- ✓ At 15.3 on Friday 14 February2003,scientists at the Dolly , the word’s most famous sheep , should be put down . she had been suffering from a progressive lung disease.

- ✓ The sheep was the mammal to be cloned using DNA taken from an adult cell


Nucleus containing source DNA
Mammary cell is extracted and grown in nutrient-deficient solution that arrests the cell cycle.
Egg cell is extracted.
Nucleus is removed from egg cell with a micropipette.
Mammary cell is inserted inside covering of egg cell.
Electric shock opens cell membranes and triggers cell division.
Preparation
Cell fusion
Cell division
Embryo
Embryo begins to develop in vitro.
Embryo is implanted into surrogate mother.
After a five-month pregancy, a lamb genetically identical to the sheep from which the mammary cell was extracted is born.
Development
Implantation
Birth of clone
Growth to adulthood

REVIEW Open Access

Handmade cloning: recent advances, potential and pitfalls

Geetika Verma[†], JS Arora[*†], RS Sethi, CS Mukhopadhyay and Ramneek Verma

Abstract

Handmade cloning (HMC) is the most awaited, simple and micromanipulator-free version of somatic cell nuclear transfer (SCNT). The requirement of expensive micromanipulators and skilled expertise is eliminated in this technique, proving it as a major revolution in the field of embryology. During the past years, many modifications have been incorporated in this technique to boost its efficiency. This alternative approach to micromanipulator based traditional cloning (TC) works wonder in generating comparable or even higher birth rates in addition to declining costs drastically and enabling cryopreservation. This technique is not only applicable to intraspecies nuclear transfer but also to interspecies nuclear transfer (iSCNT) thus permitting conservation of endangered species. It also offers unique possibilities for automation of SCNT which aims at production of transgenic animals that can cure certain human diseases by producing therapeutics hence, providing a healthier future for the wellbeing of humans. The present review aims at highlighting certain aspects of HMC including recent advancements in procedure and factors involved in elevating its efficiency besides covering the potentials and pitfalls of this technique.

Keywords: Handmade cloning, Micromanipulator, Somatic cell nuclear transfer, Transgenic, Zona free cloning

Table 1 List of animals successfully cloned by either forms of SCNT i.e. TC and HMC

S.No.	Animal	Reference(s)	
		Traditional cloning (TC)	Handmade cloning (HMC)
1.	Cattle	[78, 79]	[38, 50, 80, 81]
2.	Buffalo	[82]	[83]
3.	Goat	[84–87]	–
4.	Sheep	[1]	[73]
5.	Pig	[88]	[69, 89, 90]
6.	Horse	[91]	[92]
7.	Mice	[3]	–
8.	Cat	[93]	–
9.	Rabbit	[94]	–
10.	Rat	[95]	–
11.	Dog	[96]	–
12.	Ferret	[97]	–
13.	Wolf	[98]	–
14.	Mule	[99]	–
15.	Camel	[100]	–

porcines. All the possible mechanisms of performing HMC, in addition to its potential and limitations have been addressed besides, discussing future prospects of this technique.

Table 2 Summary of similarities and differences between HMC and TC

S.No.	Features	TC	HMC
1.	Somatic cell nuclear transfer	✓	✓
2.	Synchronization of donor cell	✓	✓
3.	High no. of attempts	✓	✓
4.	Problems associated with nuclear reprogramming and reproducibility of experiments	✓	✓
5.	True clone	×	×
6.	Micromanipulator-free	×	✓
7.	Zona- free	×	✓
8.	Problems associated with zona pellucida removal	×	✓
9.	Number of cytoplast required	Lower	Higher
10.	Costs	Higher	Lower than TC

Table 3 List of sources of donor nuclei for HMC

S.No.	Source of donor nuclei	Species	Reference(s)
1.	Pronuclear stage embryos	Mouse	[101, 102]
2.	Embryonic blastomeres	Mouse	[65]
3.	Cumulus cells	Cow	[4, 5]
4.	Embryonic stem cells (ESC)	Buffalo	[60]
5.	Adult fibroblast cells	Buffalo	[52]
		Cow	[47, 58, 103]
		Goat	[10, 12, 54]
		Sheep	[73]
6.	Natural killer T cells	Mice	[104]
7.	Fetal fibroblasts	Goat	[55, 85, 105]
		Water buffalo	[106]
		Pig	[69]
8.	Adipose tissue derived-Mesenchymal stem cells	Goat	[107]

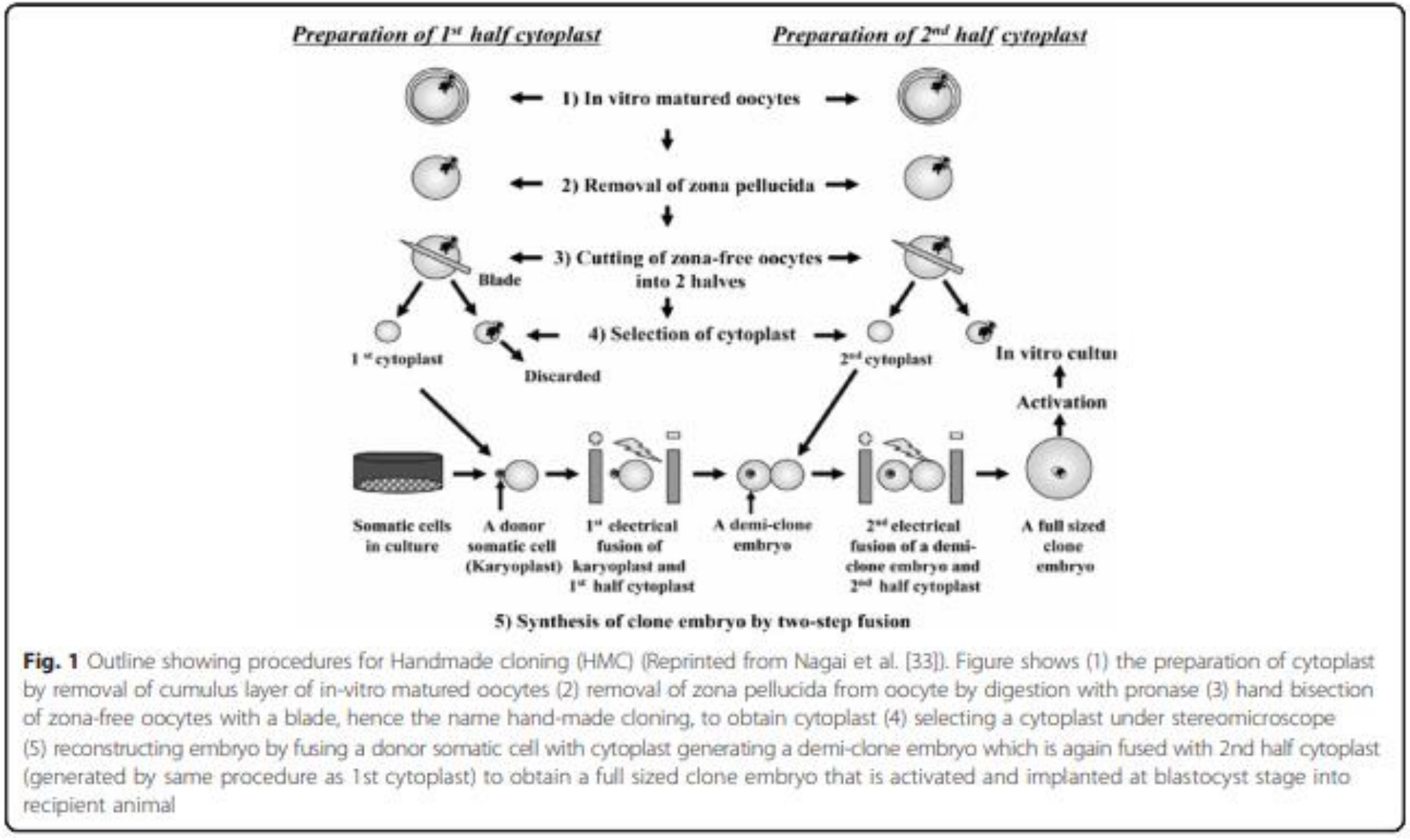


Fig. 1 Outline showing procedures for Handmade cloning (HMC) (Reprinted from Nagai et al. [33]). Figure shows (1) the preparation of cytoplast by removal of cumulus layer of in-vitro matured oocytes (2) removal of zona pellucida from oocyte by digestion with pronase (3) hand bisection of zona-free oocytes with a blade, hence the name hand-made cloning, to obtain cytoplast (4) selecting a cytoplast under stereomicroscope (5) reconstructing embryo by fusing a donor somatic cell with cytoplast generating a demi-clone embryo which is again fused with 2nd half cytoplast (generated by same procedure as 1st cytoplast) to obtain a full sized clone embryo that is activated and implanted at blastocyst stage into recipient animal

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PLOS ONE

Handmade Cloned Transgenic Sheep Rich in Omega-3 Fatty Acids

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Abstract

Technology of somatic cell nuclear transfer (SCNT) has been adapted worldwide to generate transgenic animals, although the traditional procedure relies largely on instrumental micromanipulation. In this study, we used the modified handmade cloning (HMC) established in cattle and pig to produce transgenic sheep with elevated levels of omega-3 (n−3) fatty acids. Codon-optimized nematode *mfat-1* was inserted into a eukaryotic expression vector and was transferred into the genome of primary ovine fibroblast cells from a male Chinese merino sheep. Reverse transcriptase PCR, gas chromatography, and chromosome analyses were performed to select nuclear donor cells capable of converting omega-6 (n−6) into n−3 fatty acids. Blastocysts developed after 7 days of *in vitro* culture were surgically transplanted into the uterus of female ovine recipients of a local sheep breed in Xinjiang. For the HMC, approximately 8.9% (n =925) of reconstructed embryos developed to the blastocyst stage. Four recipients became pregnant after 53 blastocysts were transplanted into 29 naturally cycling females, and a total of 3 live transgenic lambs were produced. Detailed analyses on one of the transgenic lambs revealed a single integration of the modified nematode *mfat-1* gene at sheep chromosome 5. The transgenic sheep expressed functional n−3 fatty acid desaturase, accompanied by more than 2-folds reduction of n−6/n−3 ratio in the muscle ($p<0.01$) and other major organs/tissues ($p<0.05$). To our knowledge, this is the first report of transgenic sheep produced by the HMC. Compared to the traditional SCNT method, HMC showed an equivalent efficiency but proved cheaper and easier in operation.

Dogs cloned from adult somatic cells

Two afghan pups could help to unravel the genetic behind the assorted traits of other canine breeds

Nature 436 (2005),641-642

The world's first cloned monkeys using SCNT

Zhong Zhong and
Hua Hua

Cloning in Iran

رویانا

حنا

بنیانا

تامینا

شنگول و منگول

Non-polluting cloning in some countries Allowed+

Nuclear cloning	Research cloning	country	Nuclear cloning	Research cloning	country
+	+	Japan	_	_	Austria
+	+	Singapore	_	_	Spain
_	_	France	+	+	Australia
+	+	California	_	_	Germany
_	_	Canada	_	_	Italy
+	+	Korea	_	+	Iran
_	+	Egypt	_	+	United states
_	+	Holland	_	_	Portugal
_	+	Greece	_	+	Turkey
_	+	Finland	+	+	Israel
_	+	Sweden	+	+	china

Reasons for Opposition to Cloning and their conclusion

- Violation of human dignity and concern about human production as a commodity
- The downfall of human value
- Violation of the principle of individual independence
- Violation of the principle of obtaining conscientious consent
- Violation of the ultimate goal
- Violation of principle of human perception
- Abnormal reproduction
- Randomness breach of gene combination
- The risk of racial exclusion through simulation
- Possible family foundation threat

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